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CLINICAL BIOCHEMISTRY

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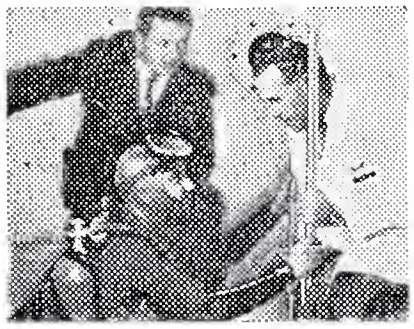

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# Guest Editorial

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1936 – *B.A. (cum laude) in Chemistry, Mount Allison University*
1941 – *Ph.D. in Pathological Chemistry from the University of Toronto*
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1946 – *Assistant Professor,* 1952 – *Associate Professor, and* 1963 – Professor *of Pathological Chemistry*
1966 *to present – Professor and Chairman of Clinical Biochemistry (the Department name was changed in* 1972)

*Won Reeve Prize for research* (1941); *was Nuffield scholar in Edinburgh and London* (1949) *and was elected a Fellow of the Royal Society of Canada* (1966).

*Has written, or co-authored, about sixty-five papers. Favourite leisure activities: sailing, tennis and skating.*

## *CLINICAL BIOCHEMISTRY, Past, Present and Future*

It is an honour, yet somehow fitting, that the Medical Society should devote an issue of its Journal to Clinical Biochemistry. The University of Toronto was among the first in the world to accord department status to this discipline and is still providing a model for similar developments, now seen as overdue in other centers. The inaugural address of the first Chairman of the Department, J. B. Leathes, was published in volume 1 of the Canadian Medical Association Journal, p. 1115, 1911, and would interest many medical students today. I quote one passage: "Every function of the body is a manifestation of ordered chemical change. Every disease is a manifestation of disordered chemical change. By determining the nature and cause of the disorder you determine the nature and cause of the disease; and till this has been determined no rational treatment of disease is possible. But, you may say, granted that this is a scientifically interesting conception, it is certainly very abstract, very remote from the practice of medicine! But what is abstract to one generation may be a well-worn implement in the mental equipment of the next."

Professor Leathes established the first biochemistry laboratory at Toronto General Hospital and founded what was probably the first Medical Research Society in Toronto, but he returned to England after war broke out in 1914. He was succeeded by Andrew Hunter, a brilliant teacher and research scientist, who relinquished the chair to Victor J. Harding in 1920 when he became Chairman of Biochemistry at Toronto and later Dean of Medicine at Glasgow University. Harding was noted for his studies of the role of electrolytes in eclampsia. In 1935, Professor Hunter returned as Chairman of Pathological Chemistry, which had moved to the Banting Institute when this building was opened in 1930. He was succeeded in 1947 by James A. Dauphinee, who guided the Department to a closer clinical orientation. Over the years the Department has played an important role in the education of medical students, in establishing Hospital clinical chemistry laboratories, and in the development of clinical investigation facilities in Toronto. Teaching functions of the Department have changed considerably in the past decade and along with research and service activities merit special comment.

Undergraduate teaching of Clinical Biochemistry occurs today in three faculties of the University – Medicine, Arts and Science, and Pharmacy. In the Faculty of Medicine 'Path. Chem.' was for many years a major subject in Third Year. There was also a laboratory course and each student served

once during the year as a 'patient' for such procedures as urea clearance, PSP, glucose tolerance or liver function tests. The day of the famous 'Pee-Derby' when, as a practical test, students were required to do a complete urinalysis on eight specimens, will be remembered only by an older generation of physicians. In spite of such requirements the course was rated highly by students.

With the restructuring of the curriculum into systems and topics, the presentation of a formal discourse on the biochemistry and physiology of human diseases was discontinued and divided into segments. In some systems the content of clinical biochemistry is more discernible and better taught than in others. A survey of student experience and opinion, if it revealed deficiencies in certain systems, could effect improvements if expressed at the level of the Period Committee, but the onus at present is on the student to fill in the gaps. Certainly there is reason to suggest that greater exposure to staff in laboratory medicine would aid the development of skills in the use of laboratory services.

Clinical Biochemistry is assuming an increasing role in graduate, and in postgraduate and continuing medical education. Up to four years of residency training can be provided leading to the Fellowship in Medical Biochemistry of the Royal College of Physicians and Surgeons. This includes a two year Diploma Course in Clinical Chemistry, available also as a Ph.D. postdoctoral training leading to Certification by the Canadian Society of Clinical Chemists. Refresher courses for clinicians, clinical pathologists and clinical biochemists are provided on a regular basis.

Research in Clinical Biochemistry incorporates skills from several disciplines in the life sciences and relates to a variety of pathological conditions. The scope ranges from the structural basis of enzyme inhibition and membrane function, to the metabolic effects of hormones and drugs, and biochemical factors in renal disorders, cancer and aging. Disease oriented basic research is essential, not only for the high standard of performance it requires, but because it is mainly through an understanding of disease at the cellular and molecular levels that major advances in medicine are going to be made. Applied research, directed to new developments and improvements in the clinical chemistry laboratories of the Hospitals, is growing slowly but steadily in output and significance. Collaboration in clinical investigation on patients has, over the years, been an important function of members of staff in the Department.

The clinical chemistry laboratory services in our Hospitals are the practical expression of all that we know about the biochemistry of disease. Responsibilities of the professional staff in these laboratories are many and varied. They are required to direct and supervise a well controlled analytical service and to be constantly innovative in providing new and improved techniques. They must be qualified to function as members of the diagnostic team of the clinician and are expected to be highly informed in all aspects of the interpretation of laboratory data in clinical biochemistry. In a teaching Hospital they must be able to develop and sustain an educational program, especially at the postgraduate professional level.

There is considerable interest in the future development of clinical chemistry services and in the manpower required to guide it. The demand for laboratory tests has increased at a rate that could be met only be automation. The expression has been formulated that:

$$\text{Clinical acumen} = \frac{\text{Complexity of the problem x Diagnostic precision achieved}}{\text{Time to reach diagnosis x Number of lab tests employed}}$$

Selective testing is much to be preferred to unreflective completeness. Since the clinician has already accepted the technology that provides unlimited data, he may be forced to accept also a computer based control of requisitioning, as well as assistance in the interpretation of tests. Commercial interests see a legitimate opportunity to exploit the system. Multiphasic screening of 'healthy' populations has generated more problems than it has benefits. The future will be challenging and exciting and the functions of the laboratory scientists cannot be allowed to become submerged by the system.

Regionalization of laboratory services will increase the need for specialists in laboratory medicine. In response to a request from government it has been suggested that one qualified clinical biochemist is required for every 300-400 active treatment beds in non-teaching hospitals, and for every 200-250 beds in teaching hospitals. In Ontario alone the manpower shortage in this field is estimated to be in excess of 100. To reach the required number of clinical biochemists within the next fifteen years will mean that about 15 post-graduate students must complete their training each year. This is about three times the present number. A portion of this group, perhaps two thirds, will have to be Ph.D.

biochemists with at least two years of postdoctoral training. In order to provide an appropriate balance of skills, approximately one third should be medically qualified.

Unfortunately, in recent years the number of first class medical graduates applying for resident positions in medical biochemistry has been small. You may ask whether reasons for this are apparent. The need for a good grounding in the sciences is probably one. Any trend to a general downgrading of basic sciences in medical curricula will surely mean that only those who enter medicine with a B.Sc. will feel themselves prepared to qualify in medical biochemistry. There is a certain ambivalence at the level of the Royal College about this, one group emphasizing the importance of clinical, the other of scientific knowledge. Our position is that the medical biochemists' first responsibility is for the quality of the laboratory service he directs. Another reason may stem from certain political-socioeconomic developments. Nearly everyone acknowledges the benefits of our system of health care. Yet in addition to being costly, it is producing certain distortions. Senior medical students perceive that it is now possible to make an attractive income in family practice in a shorter time and with less rigorous preparation than in almost any of the traditional specialties. Medical biochemistry may feel the impact of this trend most acutely because there are so few now in the field. It will not be catastrophic, but it will be regrettable, if by default this area of laboratory medicine comes to be served almost exclusively by Ph.D. clinical biochemists.

ALLAN G. GORNALL

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# *The Biochemistry and Metabolism of Plasma Lipoproteins*

*Stephen J. Wei[illegible]re, B.Sc., M.Sc., 7T5*

## INTRODUCTION

The alarming incidence of atherosclerosis and its complications in our modern society is a strong stimulus to basic research into the pathogenesis of this disease. The cornerstone of such research must be the accurate understanding of the role of plasma lipids and lipoproteins in normal physiology. Fredrickson et al[1] have reviewed what is known about the mechanisms and disorders of fat transport and have pointed to the importance of plasma lipoproteins in human disease.

A great deal of information is becoming available on the biochemistry of lipoproteins particularly related to their apoproteins. It appears that the apolipoproteins are important, not merely as the scaffold on which lipid is carried through the plasma, but as active directors of lipid metabolism. Much of this article will be devoted to a discussion of the apolipoproteins and their biochemistry, although other recent areas of interest such as the metabolism of lipoproteins and the biochemical defects in lipid disorders will also be discussed.

## BASIC CONCEPTS

The major fat transport tasks carried out in plasma are the transport of free fatty acids and triglycerides. Free fatty acids, an important metabolic fuel in the post-absorptive state, are transported bound to albumin at an estimated 25 gm. per hour during the day. Ingested fats, termed exogenous lipid, are transported as triglyceride in chylomicron particles from the intestines, via lymphatics and blood stream, to the peripheral tissues where triglyceride is removed through the action of clearing factor lipase or lipoprotein lipase. The chylomicron fragments are then cleared from the circulation by the liver.

Endogenous lipid, synthesized in the body, is transported as triglyceride via the very low density lipoproteins (VLDL). VLDL are synthesized by the liver and intestine and presumably are degraded in the peripheral tissues by lipoprotein lipase in the same manner as chylomicrons. It may be useful to regard VLDL and chylomicrons as two ends of a spectrum of triglyceride transport agents.

The role of low density lipoprotein (LDL) and high density lipoprotein (HDL) in normal physiology remains unclear. Evidence has been presented which links LDL and HDL to the catabolism of VLDL in peripheral tissues and this will be discussed in detail in a later section.

The other major lipoprotein components present in plasma are cholesterol and phospholipid. Their turnover rates are much slower than either free fatty acid or triglyceride. The extent to which lipoproteins participate in cholesterol transport has not yet been determined and for the time being we must regard cholesterol as a structural component of the triglyceride transport agents. Phospholipids are presumably present because of their surface-active properties which promote stability at oil-water interfaces and facilitate enzyme action on lipoproteins.

## THE COMPOSITION AND PROPERTIES OF LIPOPROTEINS

The composition of plasma lipoproteins is shown in Table I. Although the figures given are specific it should be stressed that there may be variation in composition within a particular lipoprotein class. Chylomicrons, for example, upon entering the blood stream from the lymphatics rapidly change composition especially with regard to fatty acids, phospholipid and protein.

TABLE I
COMPOSITION OF PLASMA LIPOPROTEINS*

| lipoprotein | protein | phospholipid | free cholesterol | cholesterol ester | triglyceride |
|---|---|---|---|---|---|
| chylomicrons | 2 | 7 | 2 | 8 | 81 |
| VLDL | 10 | 22 | 8 | 5 | 55 |
| LDL | 21 | 22 | 8 | 37 | 11 |
| HDL | 50 | 26 | 3 | 15 | 6 |

*Composition is expressed as percentage (w/w)

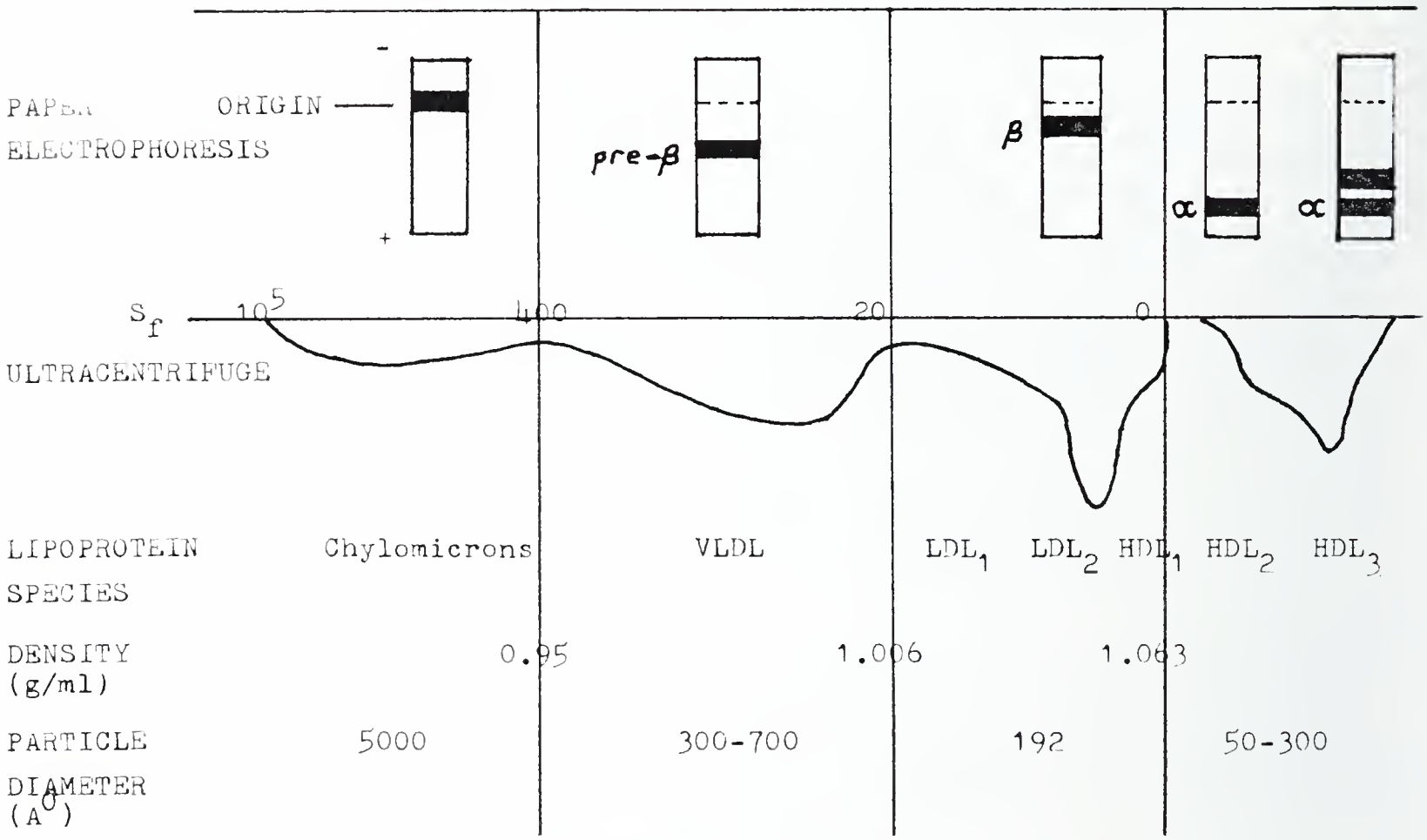
It can be seen that chylomicrons and VLDL are relatively rich in triglyceride and relatively poor in protein and phospholipid. LDL and HDL, on the other hand, have a high protein, phospholipid and cholesterol ester content but are triglyceride-poor.

Since lipoproteins contain variable amounts of lipid they are readily separated from one another by floatation in salt solutions in the ultracentrifuge. The ultracentrifugation pattern is shown in figure I along with some other physical characteristics of lipoproteins.

The lipoproteins are identified according to their density range i.e. chylomicrons $<0.95$, VLDL, 0.95-1.006, LDL, 1.019-1.063 and HDL, 1.063-1.21 g./ml. Within each density range the particles of higher density contain less triglyceride than those of lower density.

Two main subclasses of LDL are identified in the ultracentrifuge: 1) density 1.006-1.019 g./ml. and 2) density 1.019-1.063 g./ml. (figure I). The physiological significance of the two classes is not clear; however, it has been reported that subclass 2) shows better response to clofibrate administration[2]. The small shoulder on the LDL profile is labelled $HDL_1$. It is

FIGURE I
PROPERTIES OF PLASMA LIPOPROTEINS*




*Adapted from Fredrickson et al. N.E.J.M. *276*, 34, 1967.

apparently intermediate in composition and density between LDL and HDL and is not considered significant since it is not consistently present in human plasma.

There are two major subclasses of the high density lipoproteins: $HDL_2$ density 1.063-1.12 g./ml. and $HDL_3$, density 1.12-1.21 g./ml. $HDL_3$ contains more protein and has a higher ratio of cholesterol ester to free cholesterol than $HDL_2$. It has not been determined whether $HDL_3$ has physiological significance or is merely an artifact of ultracentrifugation.

The paper electrophoretic patterns of the lipoprotein classes are also shown in figure I. This technique is useful clinically to distinguish abnormal lipoprotein profiles in the hyperlipoproteinemias.

There are two abnormal lipoproteins that should be mentioned at this time. One has been described by Fredrickson et al[1] as occurring in Type III familial hyperlipoproteinemia. It is often referred to as the "broad-beta" or "floating-beta" lipoprotein demonstrating a wide beta band on paper electrophoresis and a low density (1.006 g./ml.) due to an abnormally high triglyceride content.

An abnormal lipoprotein can also be found in the plasma of patients with obstructive jaundice and this has been designated lipoprotein-X[3]. This substance has a high phospholipid content and a high ratio of free to esterified cholesterol. The protein component consists of the protein component of VLDL (apoVLDL) and albumin.

Lipoproteins are antigenic mainly by virtue of their protein components. The apoprotein of LDL (apoLDL) is an especially strong antigen and antiserum formed against this protein has been shown to react with VLDL and chylomicrons as well as with LDL suggesting the presence of apoLDL in all of these species[4]. This is borne out by the laboratory findings in cases of familial abetalipoproteinemia. These patients, who lack beta lipoprotein or apoLDL, do not have LDL, VLDL or chylomicrons in their plasma.

## LIPOPROTEIN APOLIPOPROTEINS

There appear to three main classes of apolipoproteins that can be distinguished immunochemically in human plasma. Based on this Alaupovic[5] has proposed a classification of lipoproteins into lipoprotein families according to their major apolipoprotein component. The families A, B, and C are based on those lipoproteins containing apoLP-A (HDL), apoLP-B (LDL) or apoLP-C (VLDL) as their major protein component. Thus, HDL contains mostly apoLP-A and minor amounts (less than 10% w/w) of apoLP-C. LDL contains mainly apoLP-B and minor amounts of apoLP-A and apoLP-C. VLDL contains mostly apoLP-C (55% w/w), a significant amount of apoLP-B (40% w/w) and a minor amount of apoLP-A.

The development of techniques for the delipidation of lipoproteins and the separation of apolipoproteins chromatographically has led to the isolation and characterization of peptides making up the various apolipoproteins. These peptides have been named according to their carboxyl terminal amino acid, and are listed in table II.

TABLE II
LIPOPROTEIN APOLIPOPROTEINS

| apolipoprotein | peptides isolated | molecular weight | HDL | present in LDL | VLDL |
|---|---|---|---|---|---|
| APOLP-A | apoLP-Gln-I (Thr) | 15,500 | major | minor | minor |
| | apoLP-Gln-II | 15,000 | major | minor | minor |
| APOLP-B | apoLP-Ser | 27,500 | — | major | major |
| APOLP-C | apoLP-Val | 7,000 | minor | minor | major |
| | apoLP-Glu | 10,000 | minor | minor | major |
| | apoLP-Ala | 9,800 | minor | minor | major |

Chylomicrons are extremely difficult to isolate free of contamination from other lipoprotein species. For this reason the apolipoproteins of chylomicrons have not been isolated and characterized. Amino acid analysis and immunologic techniques have suggested the presence of apoHDL and apoLDL in chylomicrons but beyond this information is lacking.

ApoHDL is known to contain at least two major peptides, apoLP-Gln-I and apoLP-Gln-II and three minor peptides, apoLP-Val, apoLP-Glu and apoLP-Ala. These minor peptides are identical to those isolated from apoLP-C and comprise 3-5% of apoHDL[6]. Until recently apoLP-Gln-I was thought to have threonine as the C-terminal amino acid and therefore, in much of the literature, is referred to as apoLP-Thr. It should be noted that these peptides were components of both $HDL_2$ and $HDL_3$, although the ratio, apoLP-Gln-I: apoLP-Gln-II, in $HDL_3$ was 3:1[6].

ApoLP-Gln-II, comprising 20-25% of apoHDL, has been shown to consist of two identical polypeptide chains connected by a disulphide bridge. Each polypeptide consists of 77 amino acid residues and has a molecular weight of 8690[7]. While no carbohydrate was found associated with apoLP-Gln-II, it is known that apoHDL contains 3% carbohydrate by weight consisting of hexose, hexosamine and sialic acid.

ApoLDL consists of one major peptide of N-terminal glutamic acid and C-terminal serine, apoLP-B, as well as minor amounts of apoLP-Ala and apoLP-Gln-I. These minor peptides represent 5-15% of the total LDL protein[8]. The heavier subclass of LDL, $LDL_2$, contains a higher proportion of apoLP-Ser than $LDL_1$. Both forms contain carbohydrate in the form of hexose, hexosamine and sialic acid.

The apolipoprotein from VLDL has been extensively characterized[9, 10]. 50% of apoVLDL consists of the three small peptides apoLP-Val, apoLP-Glu and apoLP-Ala which comprise apoLP-C. ApoLP-Ala exists in two forms designated $Ala_1$ and $Ala_2$ differing only in carbohydrate composition; apoLP-$Ala_1$ contains 1 mole of sialic acid per mole protein while apoLP-$Ala_2$ contains 2 moles of sialic acid per mole.

The amino acid sequence of apoLP-Ala has been published and showed the existence of a single carbohydrate prosthetic group bound to a threonine residue of the peptide backbone[11]. Recent work by Wetmore et al[12] has tentatively identified the prosthetic group as a disaccharide, sialyl-N-acetylgalactosamine, bound to threonine by a "mucin-type" linkage (O-glycosidic bond).

Recently a new apolipoprotein peptide of VLDL has been described representing approximately 8% of apoVLDL[13]. The molecular weight was 33,000 daltons, N-terminal glutamic acid and C-terminal sequence leu-ser-ala. The significance of this finding has yet to be determined.

## THE STRUCTURE OF LIPOPROTEINS

Chylomicrons, VLDL and LDL are found to exhibit the same general structural features. Based on these a general model of lipoprotein structure has been proposed[4]. The structure consists of an outer skin composed of a mosaic of protein, phospholipid and free cholesterol surrounding an inner core of neutral lipid and cholesterol ester. It is not known what changes in structure may accompany interaction of the lipoprotein particle with lipoprotein lipase. In some fashion, the triglyceride component of VLDL and chylomicrons must be brought into contact with the active site of the enzyme. Since we know so little about the interaction of these species it is not surprising that no lipoprotein structure to date has taken this feature into account.

While a great deal of information about the components of lipoproteins has become available, our knowledge about the integration of these components into a functioning structure is still in its infancy. With the isolation and characterization of the apolipoprotein peptides we can expect more studies on the interaction of protein and lipid to be forthcoming and these should form the basis of a detailed structure for each of the lipoprotein species.

## BIOSYNTHESIS AND METABOLISM OF LIPOPROTEINS

Early work on the biosynthesis of lipoproteins has shown that the liver is the major synthetic organ although intestinal synthesis also occurs. In vitro studies showed that radioactive amino acids could be incorporated into VLDL, LDL, and HDL by perfused rat liver and liver slices[14, 15]. On electron microscopic examination particles similar in all respects to plasma VLDL were seen in the Golgi apparatus of the rat liver cell and in the Golgi of the rat intestinal mucosal

cell[16]. These Golgi particles were shown to contain apoLDL by immunologic techniques.

These results suggested that lipoprotein synthesis was consistent with the model for the synthesis and secretion of other plasma proteins, i.e., assembly of the polypeptide backbone on the polyribosomes with migration of the peptide through channels of the endoplasmic reticulum to the Golgi apparatus. The apolipoproteins seem to be no different in this respect. It is not known, however, at what stage the lipid is added and by what mechanism? It is speculated that triglyceride may be added to the polypeptide in the channels of the smooth endoplasmic reticulum where triglyceride is formed by the esterification of free fatty acids. At any rate, completely formed VLDL particles can be seen in the Golgi apparatus with the electron microscope indicating that all components must be added by this stage.

While the exact role of the Golgi apparatus in the secretion of secretory material remains to be established, it is known that glycosylation of proteins occurs in this organelle. Presumably then, the carbohydrate moiety is transferred to the lipoprotein apoproteins in this region. The function of the carbohydrate remains obscure.

The synthesis of apolipoproteins by isolated, perfused rat liver and intestine was recently investigated[17]. Both preparations were capable of incorporating labelled amino acids into secreted VLDL and HDL. These lipoproteins were secreted into the perfusate by liver and into the lymph by intestine. It is interesting that there was no label found in the small peptides, apoLP-Glu and apoLP-Ala of intestinal VLDL. It was suggested that intestinal VLDL must have acquired preformed apoLP-Glu and apoLP-Ala on secretion into the lymph. These peptides could have been transferred from circulating HDL since it is known that the peptides apoLP-Val, apoLP-Glu and apoLP-Ala transfer freely between VLDL and HDL in vivo[18]. Further experiments on the interrelationship of lipoproteins should give a definitive explanation of the above observation.

Currently a controversy exists as to whether LDL is secreted by the liver or is derived in plasma from the catabolism of VLDL[8]. The latter view, which is currently popular, is based on the following suggestive evidence: 1) While VLDL particles have been visualized with the electron microscope in the Golgi apparatus of liver cells, LDL particles have never been seen there. 2) Calculations of VLDL and LDL turnover in the plasma have shown that all LDL can be accounted for by VLDL breakdown. 3) The cholesterol and phospholipid of VLDL as well as the apoLDL portion of VLDL appear to decay through an intermediate lipoprotein of density 1.006-1.019 g./ml. and then into LDL. Thus, VLDL is broken down in the plasma by a two-step process ending ultimately in LDL[19]. The first step in this process is dependent on lipoprotein lipase activity.

The small peptides of VLDL, apoLP-Val, apoLP-Glu and apoLP-Ala, have been shown to readily exchange between VLDL and HDL in vivo and in vitro[18]. When heparin is infused into the blood stream resulting in release of lipoprotein lipase into the plasma and hydrolysis of VLDL, there is a gross increase in the protein of HDL due to a transfer of these small peptides from VLDL. Protein labelling studies have shown that within 6 hours the small peptides may be transferred back to VLDL[8]. Recently it was discovered that one of these peptides, apoLP-Glu, was an activator of the enzyme lipoprotein lipase[20].

Based on these observations of lipoprotein interrelationships a picture of lipoprotein metabolism is beginning to emerge and may be summarized as follows: VLDL is synthesized and secreted by liver and intestine. In the capillaries of adipose tissue VLDL interacts with lipoprotein lipase. The enzyme may be activated by the protein component of VLDL specifically the peptide, apoLP-Glu, and VLDL is hydrolysed. As a result triglyceride is transferred into the adipose tissue and small peptides are transferred to HDL leaving a particle of intermediate density which quickly decays to native LDL. HDL may act as a vehicle for transporting the small peptides in the circulation back to liver and intestine where the peptides may be recycled into VLDL. In contrast, all the evidence indicates that LDL is an end stage in the plasma and its protein is not recycled into any other lipoprotein[8].

One other aspect of lipoprotein metabolism that should be discussed is the role of cholesterol and cholesterol ester. Free cholesterol appears to be freely exchangable in plasma, rapidly equilibrating between the lipoprotein classes. The exchange between free and esterified cholesterol occurs because cholesterol esters are hydrolyzed in the liver contributing free cholesterol to the plasma cholesterol pool. The cholesterol esters are believed to be synthesized in the plasma by the enzyme, lecithin: cholesterol acyl transferase, (LCAT), which transfers a fatty acyl residue from lecithin to cholesterol giving lysolecithin and cholesterol ester. The LCAT enzyme was found to associate primarily with the HDL fraction of human plasma[21] although LDL cholesterol was also a substrate for this enzyme.

While the role of cholesterol, cholesterol ester and LCAT in lipoprotein function remains very vague, two suggestions merit discussion. These are: 1) that the LCAT mechanism is the means by which cholesterol in the peripheral tissues (primarily in cell membranes), is picked up, esterified to cholesterol ester and transported via HDL and perhaps LDL to the liver for use in that organ[21] and 2) that cholesterol ester may be transferred from HDL and perhaps LDL to VLDL in exchange for

triglyceride[22]. The cholesterol ester of VLDL in some way helps to stabilize this particle. This may be the mechanism by which VLDL obtains cholesterol ester in vivo since its free cholesterol component does not appear to be very active in the LCAT reaction.

## BIOCHEMICAL AND METABOLIC DEFECTS IN LIPID DISORDERS

As the picture of lipoprotein metabolism develops, some insight can be gained into the basic biochemical defects resulting in disorders of lipid metabolism. The clinical features and treatment of these disorders have been well described[1] and will not be discussed here. A suitable reference for most of the material contained in this section is included in the reference list[23].

*Abetalipoproteinemia* – The present evidence indicates that the defect in this disease is the selective absence of apoLDL, (apoLP-Ser), resulting in the absence of chylomicrons, VLDL and LDL from the patient's plasma. While the lipoprotein particles themselves are not present, the peptides of apoVLDL and apoHDL can be found and are apparently normal in structure. Furthermore, no abnormal apoLDL peptide can be found in the plasma indicating that the absence of apoLDL is due to a failure to synthesize this peptide. HDL is the only lipoprotein present although in lower concentration than in normals.

*Familial Hypobetalipoproteinemia* – This disease is apparently unrelated to abetalipoproteinemia. The basic finding in these patients is that plasma LDL levels are ten times lower than normal controls and that VLDL and chylomicrons are correspondingly reduced. The LDL and apoLDL isolated from these patients were chemically and immunologically identical to LDL and apoLDL from normals; however, the rate of entry of new LDL into the circulation of these patients was 10% of the normal rate. The reason for this reduced synthetic rate of LDL remains to be determined. It is possible that some minor structural abnormality in apoLDL or a defect in the assembly of lipoprotein particles in the liver is responsible. It is interesting that patients with this disease retain the ability to respond to high carbohydrate feeding by an increase in VLDL secretion.

*Familial HDL Deficiency* – In this rare disorder, also called Tangier disease, normal plasma HDL is absent and is replaced by low concentrations of a mutant HDL (HDLT). This mutant form has been isolated, characterized, and shown to contain an abnormal ratio of apoLP-Gln-I to apoLP-Gln-II. In contrast to normal HDL which has an apoLP-Gln-I: apoLP-Gln-II ratio of 3:1, only trace amounts of apoLP-Gln-I are found in HDLT. This small quantity of apoLP-Gln-I appears to be identical in structure to the peptide of normal HDL. Thus, the defect in Tangier disease may be located in the gene controlling synthesis of apoLP-Gln-I while normal synthesis of apoLP-Gln-II occurs.

One clinical finding that has yet to be explained in Tangier disease is the deposition of cholesterol ester in reticuloendothelial cells forming the swollen, orange tonsils characteristic of this disease. This may be of significance in relation to the mechanism of cholesterol and cholesterol ester deposition in atheromatous plaques. Unfortunately, there is little to offer but suggestion at this time. One suggestion is that the abnormal HDL of Tangier disease may affect the LCAT reaction resulting in a reduction in cholesterol ester formation and a subsequent accumulation of free cholesterol in the tissues. Since cholesterol ester formation was reduced there would be a failure to transfer cholesterol ester from HDL to VLDL in the plasma, resulting in unstable VLDL particles. These could be taken up by macrophages of the reticuloendothelial system leading to lipid accumulation in this tissue.

Recently it was discovered that the peptide apoLP-Gln-I was an activator of the LCAT enzyme[24]. The lack of this peptide in Tangier disease makes it highly likely that a deficiency in LCAT activity is important in the pathogenesis of this disease.

*Familial Lecithin: Cholesterol Acyl Transferase Deficiency* – This very rare disease has so far been found in only seven people, members of three Scandanavian families. It has been demonstrated that the plasma from these patients lacked LCAT activity and that this was due to an absence of LCAT enzyme per se, rather than due to the presence of some LCAT inhibitor or the failure of lipoproteins to act as LCAT substrates. As expected from the known actions of LCAT, the plasma lipoproteins in this disorder showed an increase in free cholesterol and lecithin and a decrease in cholesterol ester and lysolecithin. There was some preliminary evidence to suggest that an HDL fraction may be secreted by the liver of these patients that is analogous to newly secreted HDL in normals. It may be that this newly secreted HDL fraction fails to mature to normal HDL in patients who lack LCAT activity since cholesterol ester is not formed. Accordingly, due to mechanisms previously discussed, VLDL and LDL will be of abnormal structure.

It was also observed that VLDL and HDL from LCAT-deficient patients showed abnormal electrophoretic mobility; VLDL migrated to the beta position instead of pre-beta and some HDL migrated to the alpha$_2$ position instead of alpha$_1$. In view of the close association of LCAT and HDL it was speculated that the defect responsible for the absence of LCAT also resulted in the production of an abnormal apolipoprotein of HDL accounting for the unusual electrophoretic mobilities of the VLDL and HDL. Isolation and characterization of the apolipoproteins from LCAT-deficient patients should shed some light on the basic defect in this disease.

*Familial Hyperlipoproteinemia – Type I* – This

disease is characterized by an increase in the chylomicron content in plasma from patients maintained on a normal diet. The basic defect is a failure to remove chylomicrons from the plasma due to the absence or deficiency of the enzyme lipoprotein lipase. This can be documented by finding a low plasma lipase activity following heparin infusion. In fact, most of the observed post-heparin lipase activity is due to a hepatic triglyceride lipase, distinct from lipoprotein lipase, that is also released by heparin infusion.

Thus, the defect in this disease appears to be the selective absence of lipoprotein lipase activity. The possibility that a defective enzyme activator was responsible for the absence of LCAT was ruled out by the finding that the small peptides of VLDL, apoLP-Val, apoLP-Glu and apoLP-Ala, were normal in amount and structure and further, that apoLP-Glu was capable of activating lipoprotein lipase in vitro.

A paradox that has not been explained is why there was no accumulation of VLDL in the plasma of these patients since chylomicrons and VLDL are both degraded by lipoprotein lipase? It may be that other means of removal of VLDL are in operation, such as phagocytosis of the excess particles. At any rate, knowledge about the structure of chylomicrons and their apoproteins is awaited in order to fully investigate the interaction of these lipoproteins and lipoprotein lipase.

*Familial Hyperlipoproteinemia – Type II* – This relatively common disorder is characterized by an increase in LDL concentration in plasma and is often referred to as hyperbetalipoproteinemia. There are two subclasses, Type IIa and Type IIb according to whether the VLDL concentrations are normal (IIa) or high (IIb). The subclass of LDL that is primarily elevated in this disease is $LDL_2$ (see figure I).

The exact nature of the biochemical defect causing increased plasma LDL is unknown. Investigations of LDL isolated from patients with this disease have shown that the apoprotein, consisting mainly of the peptide apoLP-Ser, was normal in structure, conformation and immunochemical behaviour. Also there appeared to be no abnormalities in either the lipids present or the lipid:protein ratio in the LDL isolated from these patients.

It has been shown by labelling studies that there was a normal synthetic rate of LDL but a depressed catabolic rate in Type II patients. Presumably LDL is normally degraded in the liver; however, as the exact details of this reaction are lacking, no specific defect can be pinpointed. Further advances in knowledge about this disease await elucidation of the normal mechanism of LDL catabolism.

*Familial Hyperlipoproteinemia – Type III* – The presence of a "floating-beta" VLDL species in plasma characterizes this disease. It is apparently due to an abnormally high triglyceride content of the VLDL particle. Investigations of the beta VLDL by immunochemical methods have shown that the apoprotein contained apoLDL, (apoLP-Ser), but did not contain the small peptides of apoVLDL, apoLP-Val, apoLP-Glu and apoLP-Ala. Furthermore, the apoLDL appeared to be identical with the apoLDL from normal subjects.

The catabolism of VLDL in normal plasma was discussed in a previous section. It was held that VLDL was degraded in a two-step mechanism through an intermediate lipoprotein to LDL. The first step was catalysed by lipoprotein lipase and resulted in the small peptides of VLDL being transferred to HDL. It is interesting to speculate that the "floating-beta" VLDL observed in Type III disease is the postulated intermediate lipoprotein present in the plasma because of a defect in the second step of VLDL catabolism. This would imply that the "floating-beta" or intermediate lipoprotein was present in human plasma normally but was degraded to LDL so rapidly as to be undetectable. It would also be consistent with the observed composition of the "floating-beta" VLDL apoprotein, i.e. presence of normal apoLDL but absence of the small peptides of apoVLDL.

The physiological significance of this mechanism for VLDL catabolism remains controversial and likewise, the nature of the defect resulting in the observed lipoprotein abnormality remains unknown.

*Familial Hyperlipoproteinemia – Types IV and V* – These two diseases are included together because of the similarities in their lipoprotein patterns. In both cases there is an increased level of VLDL in the plasma and in Type V, an increase in chylomicrons is seen as well.

Unfortunately, they are also included together because very little is known about the cause of these disorders. To date no abnormalities in lipoprotein structure have been found; however, this must be tempered by the fact that knowledge of the structural components of chylomicrons is severely lacking. It may be that minor abnormalities in structure of the apolipoproteins of either chylomicrons or VLDL may be of maior significance in their metabolism in view of the activity of some of these peptides as enzyme cofactors.

Other possibilities for the defects in these diseases must be borne in mind. These include the control of glyceride synthesis and catabolism and the hormonal influence on enzymes such as lipoprotein lipase.

## CONCLUSION

Lipoproteins are complex particles dedicated to the transport of triglyceride through human plasma. A great deal of information exists about the structure and function of the lipoprotein components that enable

the above metabolic task to be carried out. Unfortunately knowledge about the integration of these components into a functioning structure is lacking.

While the accumulation of such knowledge is a tedious job carried out by dedicated researchers, it should be appreciated that such basic information is an essential foundation on which the study of disease mechanisms can be built. It should also be evident from this article that the more we know, the more we need to know, for, although we have gained some knowledge of the biochemistry of plasma lipoproteins, we are still groping in the dark as far as the biochemical and metabolic defects of the lipid disorders are concerned.

REFERENCES


1. Fredrickson, D. S.; Levy, R. I.; Lees, R. S.: New Eng. J. Med. *276,* 34, 94, 148, 215, 273, 1967.
2. Strisower, E. H.; Adamson, G.; Strisower, B.: Am. J. Med. *45,* 488, 1968.
3. Seidel, D.; Alaupovic, P.: Dt. Med. Wschr. *95,* 1174, 1870.
4. Schumaker, V. N.; Adams, G. H.: Ann. Rev. Biochem. *38,* 113, 1969.
5. Fredrickson, D. S.; Gotto, A. M.; Levy, R. I., in THE
5. Alaupovic, P., Atherosclerosis *13,* 141, 1971.
6. Scanu, A. M. in PLASMA LIPOPROTEINS, Biochemical Society Symposia: No. 33, edited by R. M. S. Smellie, pp. 29-45, Academic Press, London, 1971.
7. Lux, S. E.; John, K. M.; Brewer, H. B. Jr.: J. Biol. Chem. *247,* 7510, 1972.
8. Lewis, B. in PLASMA LIPOPROTEINS, Biochemical Society Symposia: No. 33, edited by R. H. S. Smellie, pp. 19-28, Academic Press, London, 1971.
9. Brown, W. V.; Levy, R. I.; Fredrickson, D. S.: J. Biol. Chem. *244,* 5687, 1969.
10. Brown, W. V.; Levy, R. I.; Fredrickson, D. S.: J. Biol. Chem. *245,* 6588, 1970.
11. Brewer, H. B. Jr.; Shulman, R.; Herbert, P.; Ronan, R.; Wehrly, K.: Adv. Exp. Med. and Biol. *26,* 280, 1972.
12. Wetmore, S.; Mahley, R. W.; Brown, W. V.; Schachter, H.: Fed. Proc. *32,* 524, (Abst.), 1973.
13. Shelburne, F. A.; Quarfordt, S. H.: Fed. Proc. *32,* 1853 (Abst.), 1973.
14. Roheim, P. S.; Miller, L.; Eder, H. A.: J. Biol. Chem. *240,* 2994, 1965.
15. Lo, C.; Marsh, J. B.: J. Biol. Chem. *245,* 5001, 1970.
16. Mahley, R. W.; Hamilton, R. L.; LeQuire, V. S.: J. Lipid Research *10,* 433, 1969.
17. Windmueller, H. G.; Herbert, P. N.; Levy, R. I.: J. Lipid Research *14,* 215, 1973.
18. Bilheimer, D. W.; Eisenberg, S.; Levy, R. I.: Biochem. Biophys. Acta *280,* 94, 1972.
19. Bilheimer, D. W.; Eisenberg, S.; Levy, R. I. Biochem. Biophys. Acta *260,* 212, 1972.
20. LaRosa, J. C.; Levy, R. I.; Herbert, P.; Lux, S. E.; Fredrickson, D. S.: Biochem. Biophys. Res. Commun. *41,* 57, 1970.
21. Glomset, J. A.: J. Lipid Research *9,* 155, 1968.
22. Norum, K. R.; Gloset, J. A.; Gjone, E. in THE METABOLIC BASIS OF INHERITED DISEASE, edited by J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrickson, 3rd ed., p. 538, McGraw-Hill, New York, 1972.
23. THE METABOLIC BASIS OF INHERITED DISEASE, edited by J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrickson, 3rd. edition, chapters 26, 27, 28, McGraw-Hill, New York, 1972.
24. Fielding, C. J.; Shore, V. G.; Fielding, P. E.: Biochem. Biophys. Res. Commun. *46,* 1493, 1972.



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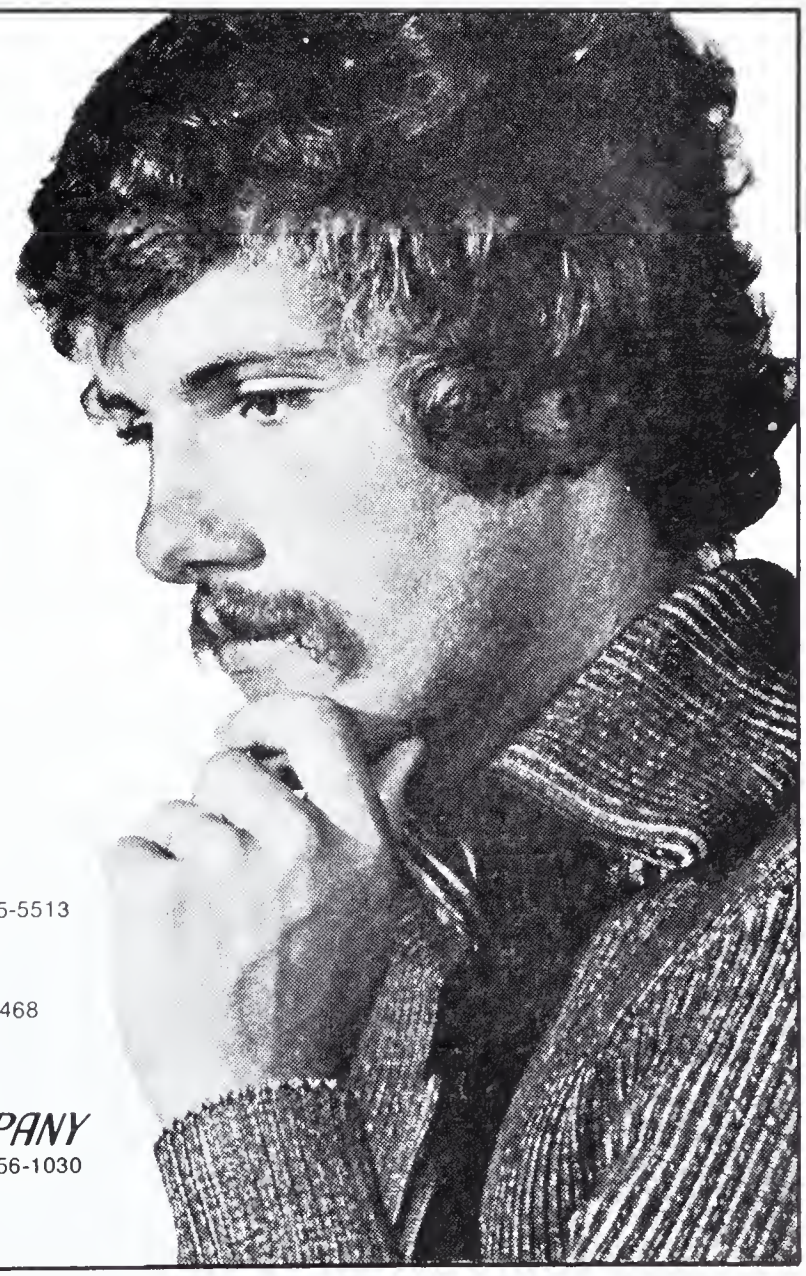


# *Biochemical Investigations of Amniotic Fluid in the Estimation of Foetal Maturity*

*Jeffrey A. Nisker*, 7T4

PREAMBLE

It is becoming increasingly important in the practice of modern obstetrics to establish the means of an accurate in utero assessment of foetal maturity.

Frequently, when attending high-risk obstetrical cases, the obstetrician is presented with clinical situations that may indicate early delivery of the foetus. This situation may arise when confronted with Rh – isoimmunization, maternal diabetes mellitus, toxemia of pregnancy, maternal heart disease, a history of previous classical Caesarian section, and other problems affecting mother and/or foetus. This practice of early delivery is associated with increased neonatal mortality due to the immature organ systems of the foetus not yet capable of supporting the newborn in an extra-uterine environment. Since up to 75% of neonatal deaths are attributed to immature lungs[1] and the resulting Respiratory Distress Syndrome, foetal lung maturity, in particular, requires accurate evaluation.

Thus, although the maternal clinical status may indicate the termination of her pregnancy, early delivery may jeopardize foetal survival. The obstetrician is thus in a difficult position. As he continues to delay the initiation of early delivery, he is allowing more time for the foetal organs (especially the lungs) to mature, but he also is allowing the risk of maternal mortality to continue. Immediate delivery would remove the threat to the mother but may condemn the foetus to R.D.S. and probable death.

It is because of this critical situation, which is occurring now not infrequently, that methods to accurately determine the level of foetal maturity in utero, must be perfected and made readily available to the obstetrician. The obstetrician has an accurate appraisal of maternal status; but only when in utero estimations of foetal status become dependable, will the obstetrician have the information he needs on which to base his decision whether to proceed with or defer early delivery.

There is an increasing number of guidelines to help the obstetrician determine this optimum time of delivery.

The traditional guideline is the expected date of confinement (E.D.C.), as calculated by Naegele's rule, from the first day of the last menstrual period. This E.D.C. is given more substance by clinical evaluation of uterine size. If the foetus is maturing at a normal rate, gestational age can be correlated with the degree of foetal maturity. The E.D.C. is often difficult to predict by Naegle's rule, for many reasons. A study by Underhill and Beazley in 1971[2] suggested that this difficulty may extend to approximately 22% of patients and that E.D.C. is generally a very inaccurate criterion of foetal maturity, especially in the presence of any complication of pregnancy.

Radiological observation of the foetus, especially the area of distal femoral epiphysis, may be indicative of foetal maturity in uncomplicated pregnancies. Repeated exposure to x-ray, however, has recently been implicated as a predisposing factor in children susceptible to leukemia[3] and thus may not be as innocuous as once assumed.

The measurement of the biparietal diameter by ultrasonography is becoming a highly respected technique for the estimation of foetal weight and gestational age. Unfortunately, the limited number of skilled technicians and the expense of the apparatus makes this procedure unavailable to many physicians.

Since 1965 the most energetic search for an accurate method of determining foetal maturity has been directed toward biochemical and cytological anaylsis of amniotic fluid. Several parameters can now be studied: bilirubin concentration, creatinine concentration, percentage of fat-laden cells and phospholipid quantity. These determinations allow empirical assessment of foetal maturity.

Investigations of bilirubin concentration give an indication of liver maturity and the presence of H.D.N. Creatinine studies reflect kidney maturity. Techniques that allow the calculation of the percentage of fat-laden cells in the amniotic fluid indicate the maturity of the sebaceous glands, and phospholipid studies give information concerning the maturity of the foetal lungs.

As noted above, it seems that the status of the foetal lungs is of primary importance in the prediction of neonatal survival[1]. Therefore, this paper will discuss in greatest detail the phospholipid studies and outline more briefly the other biochemical investigations of the amniotic fluid.

It is first necessary to consider the method of obtaining amniotic fluid for biochemical investigation.

AMNIOCENTESIS

Amniotic fluid may be obtained by two routes: transvaginal and transabdominal. The vaginal approach is prone to contamination and other sources of error and is now used very infrequently. It will not be discussed further in this review.

The transabdominal approach, although by no means infallible, is more accurate. As it was the method used in most of the studies reviewed for this paper, it will be described further.

The procedure of transabdominal amniocentesis had its beginnings in the late 1920's, at which time it was employed almost exclusively as a method for retrieving amniotic fluid for studies of reproductive physiology. When infections following this procedure were cited as the cause of several foetal and maternal mortalities, the method was quickly abandoned.

Transabdominal amniocentesis next surfaced in Japan in the late 1940's, where this controversial technique was employed as the vehicle for hypertonic saline infusion in the termination of pregnancies of greater than twelve weeks gestation[4].

However, it was not until 1950 when Bevis[5] used transabdominal amniocentesis to obtain amniotic fluid for his historic studies on amniotic fluid bile pigment and its relation to foetal involvement in cases of Rh – isoimmunization, that this technique gained respectability. These studies were immediately followed by many similar investigations, and after numerous reports confirmed the reliability and safety of the transabdominal method, it soon became an important investigative technique in the armamentarium of the obstetrician.

Before performing transabdominal amniocentesis, it is recommended that an ultrasound (B-scan) picture be obtained in order to localize the placenta. Such localization is very important, as an anterior lying placenta may be easily punctured if the obstetrician is unaware of its position. Entry into the placenta, even if the needle passes completely through its substance, will not yield amniotic fluid capable of accurate analysis and may lead to foetal death. Thus, when an anterior lying placenta is discovered, the amniocentesis should be done with continuous ultrasonography guiding the needle superior or inferior to the placenta. This is not to suggest that amniocentesis is unsafe without ultrasound scans, but rather to point out the advantage of using ultrasound when possible.

Also, before amniocentesis is performed the patient should void, to lessen the danger of bladder perforation. Finally, before the patient is prepared the foetal heart tone must be confirmed.

The skin surface through which the needle will be passed is prepared with a suitable antiseptic. The suggested areas through which the needle may be introduced are either suprapubically in the mid line (if the head can easily be ballotted up), or higher in the abdomen on the small parts side approximately two inches below and two inches lateral to the umbilicus. There is currently much debate on which of these approaches is more successful and produces less morbidity, but it is not in the scope of this paper to discuss it further.

A local anaesthetic is injected, first raising a surface skin wheal, and then penetrating deeper to anaesthetize a tract towards the uterus. A 20-gauge spinal needle is then introduced through the anaesthetized tissue and uterine wall into the amniotic fluid-filled space. Next, 10 ml. of fluid is withdrawn. If the fluid appears bloody, another spinal needle should be introduced approximately 3 cm. laterally. Bloody fluid does not yield accurate analyses.

## CYTOLOGIC INVESTIGATION

Although the most important investigations of amniotic fluid employ biochemical techniques, cytological techniques are also important. A description of a cytological investigation is included for completeness.

The most important cytological test is the quantitative analysis of foetal fat-laden cells. A correlation between these fat-laden cells and foetal maturity appeared in the literature in 1967 with the Gordon and Brosen study[6]. They made the observation that fat-laden cells exfoliated from maturing feotal sebaceous glands stain orange when treated with Nile blue sulfate in a prescribed manner[6, 7]. By calculating the percentage of amniotic fluid cells that stain orange and comparing these figures with those accepted as the mean for each stage of gestation, they were able to estimate foetal maturity.

It is generally accepted that if greater than 10% of foetal cells are orange-staining an estimation of gestational age of greater than 36 weeks can be made. It must be noted that the Kling study[7] showed 42% false positives and that other studies have appeared, supporting Kling's conclusion that although the Nile blue sulfate test is a fast and simple method of estimating foetal maturity, it is grossly inaccurate when compared to biochemical studies. Therefore, this test must only be used as an adjunct to other methods of estimating foetal maturity, and its results should not be considered accurate if it is the only parameter available.

## BIOCHEMICAL INVESTIGATION

### *Bilirubin levels*

Bilirubin is a pigment formed as a breakdown product by hemolysis of erythrocytes. Bilirubin is produced in small amounts by the foetus due to the normal maturation and death of red cells, but may be produced in much larger amounts if a hemolytic process, such as Rh – isoimmunization is occuring in utero.

The bilirubin found in amniotic fluid analysis is in the unconjugated form. This would be expected as the foetal liver does not possess substantial quantities of glucuronyl transferase, an enzyme required for bilirubin conjugation. It is essentially the maturation of this enzyme system causing lower levels of unconiugated bilirubin as pregnancy proceeds that makes bilirubin levels of significance in estimating foetal maturity.

There are two major techniques of measuring

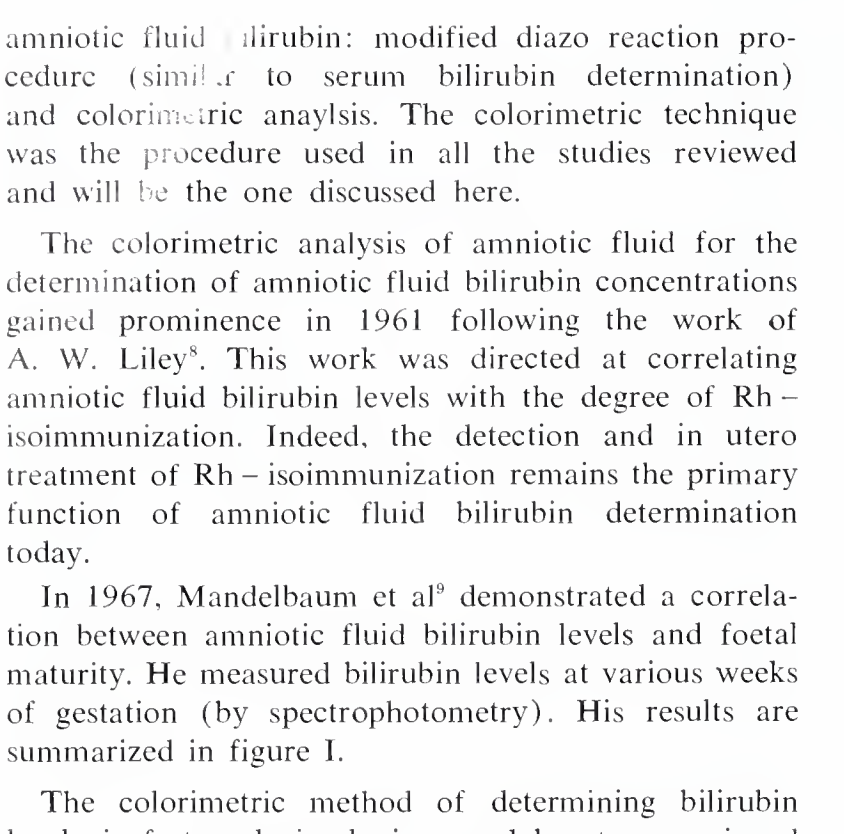
amniotic fluid bilirubin: modified diazo reaction procedure (similar to serum bilirubin determination) and colorimetric anaylsis. The colorimetric technique was the procedure used in all the studies reviewed and will be the one discussed here.

The colorimetric analysis of amniotic fluid for the determination of amniotic fluid bilirubin concentrations gained prominence in 1961 following the work of A. W. Liley[8]. This work was directed at correlating amniotic fluid bilirubin levels with the degree of Rh – isoimmunization. Indeed, the detection and in utero treatment of Rh – isoimmunization remains the primary function of amniotic fluid bilirubin determination today.

In 1967, Mandelbaum et al[9] demonstrated a correlation between amniotic fluid bilirubin levels and foetal maturity. He measured bilirubin levels at various weeks of gestation (by spectrophotometry). His results are summarized in figure I.

The colorimetric method of determining bilirubin levels is fast and simple in any laboratory equipped with a spectrophotometric apparatus. 3 ml. of centrifuged amniotic fluid is transferred to a colorimetric tube and read at 10 nm increments from 350-700 nm in any standardized spectrophotometer. The results are plotted on semilogarithmic paper and the bilirubin content is then estimated from the optical density at 450 nm as described by Liley[8]. The data obtained at various periods of gestation are shown in figure I. It must be remembered that if blood or meconium is present in the amniotic fluid, or if the fluid is exposed to direct sunlight, accurate analysis is impossible.

FIGURE I




Typical decrease of the 450 nm peak in serial spectrophotometric analyses as pregnancy progressed in one patient. The peak was large in early pregnancy, but disappeared at 36 weeks. (From Mandelbaum, B., [9]).

Thus bilirubin levels in the amniotic fluid normally decrease as gestation proceeds and if no Rh – isoimmunization is present there is only a slight shoulder on the curve at the 450 nm wave length.

It has been suggested by studies of Mandelbaum et al[9] and those of other investigators that when the optical density of amniotic fluid is less than 0.010 at 450 nm one may estimate the foetal maturity as normal for 34 weeks gestational age. Most comparative studies, including the study of Kling et al[7], indicate that amniotic fluid bilirubin levels do not predict foetal maturity as accurately as creatinine or phospholipid studies. Bilirubin also is useless as a parameter of foetal maturity if Rh – isoimmunization is present or anticipated. This is obvious, as the great quantity of unconjugated bilirubin in the amniotic fluid of the isoimmunized foetus will consistently give large peaks at 450 nm and lead to gross false negative interpretations.

*Creatinine concentration*

Amniotic fluid creatinine concentration became a prominent parameter of foetal maturity following the study by Pitkin and Zwirek[10], published in 1967. Many studies, including an excellent one by Doran et al[11] at the Toronto General Hospital, have supported the results of their work.

The method generally employed to determine amniotic fluid creatinine concentration[7] includes: deproteinization of the amniotic fluid by the addition of 3 ml. distilled water, 1 ml. 10% sodium tungstate, and 2 ml. 2/3 N Sulphuric acid to every 2 ml. of amniotic fluid, and thoroughly mixing the solution. The deproteinized amniotic fluid is then assayed by the Jaffé picric acid method[21] or the Folin-Wu technique[13].

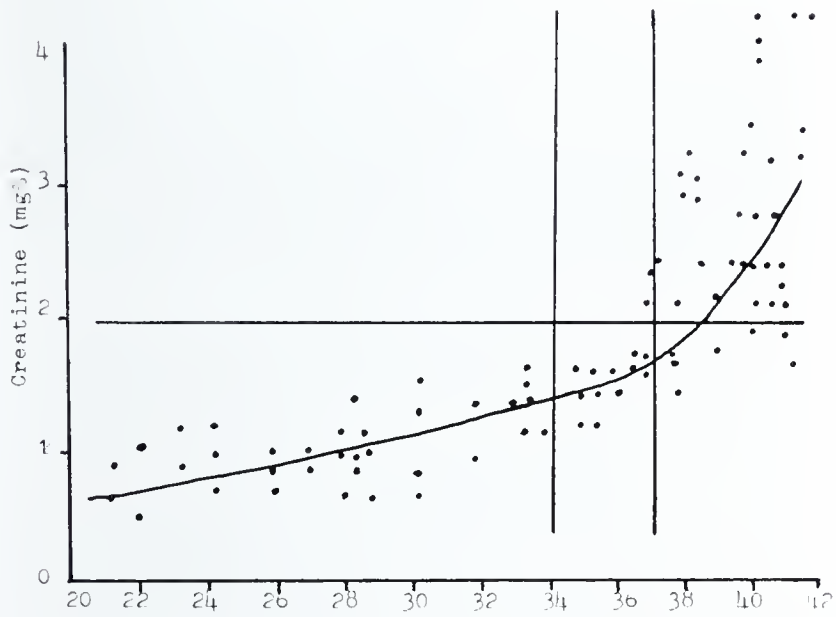
The data of Pitkin and Zwirek[10] (figure II) show that the concentration of creatinine increases very gradually as gestation progresses, up to 34 weeks. At 34 weeks a more marked increase occurs so that by 37 weeks the creatinine concentration exceeds 2 mg% in 94% of cases. The increased volume of foetal urine in amniotic fluid at this time of gestation seems the most probable explanation for the rising creatinine levels.

The relationship between a gestational age of 37 weeks or greater and an amniotic fluid creatinine concentration of 2 mg. per cent or more is significant by chi square analysis, ($P=0.001$).

Other studies support the assertion that amniotic fluid creatinine concentrations of 2 mg% or greater appear to be a reliable index of foetal maturity. It has also been shown that the creatinine concentration is not affected by the common diseases complicating pregnancy, such as diabetes mellitus, preeclampsia, chronic hypertension, and Rh – isoimmunization[10]. The presence of one of these diseases is often the reason

why the obstetrician wants to deliver prematurely and therefore must have knowledge of foetal maturity. The fact that creatinine concentration as an index of foetal maturity is not affected by these complications makes it a worthwhile test indeed.

FIGURE II




Mean results of single random determinations of creatinine in amniotic fluid. Pitkin and Zwirek[10].

*Phospholipid Studies*

In 1957, Clements et al[14] identified the presence of material called "surfactant" in lung tissue. This substance was later demonstrated to lower the alveolar surface tension thus allowing the alveoli to expand and fill more readily. Brumley et al[15] found that when comparing the lungs of infants whose mortality was due to the immature lung syndrome with the lungs of infants that died of other causes, there were consistently lower surfactant levels associated with the immature lung deaths. It was known that the immature lung syndrome occurred almost always in premature and immature babies. It thus became evident that if the amount of surfactant in the lungs could be determined in utero it would do much to predict which foetus if delivered prematurely, either spontaneously or by obstetrical intervention, would develop the immature lung syndrome. Because the fluid drift from foetal lung to amniotic fluid cavity carries surfacant with it, it was postulated and later proven that the concentration of surfactant in amniotic fluid is a good indication of the amount of surfactant coating the alveoli[16].

Surfactant is a complex of protein, carbohydrate, and lipid. The main lipid is dipalmitoyl phosphatidyl choline, also known as lecithin. Lecithin has a hydrophobic portion due to its fatty acid and a hydrophilic portion where the choline group is attached and this structure is probably what enables this phospholipid to lower the surface tension on alveoli. The work of T. E. Morgan[17] indicated the source of lecithin to be inclusion bodies within alveolar type II cells.

Sphingomyelin is another phospholipid found in the amniotic fluid. It does not seem to play an important role in lessening alveolar surface tension. The sphingomyelin concentration of amniotic fluid stays at a relatively constant level as gestation progresses and serves as an excellent parameter against which amniotic fluid lecithin concentrations may be compared. Thus the L/S ratio is a good indication of the amount of surfactant present as pregnancy proceeds to term.

Two methods of phospholipid determination will be discussed here. The first is the standard method, employing thin layer chromatography as described by Gluck[16] and Kling[7].

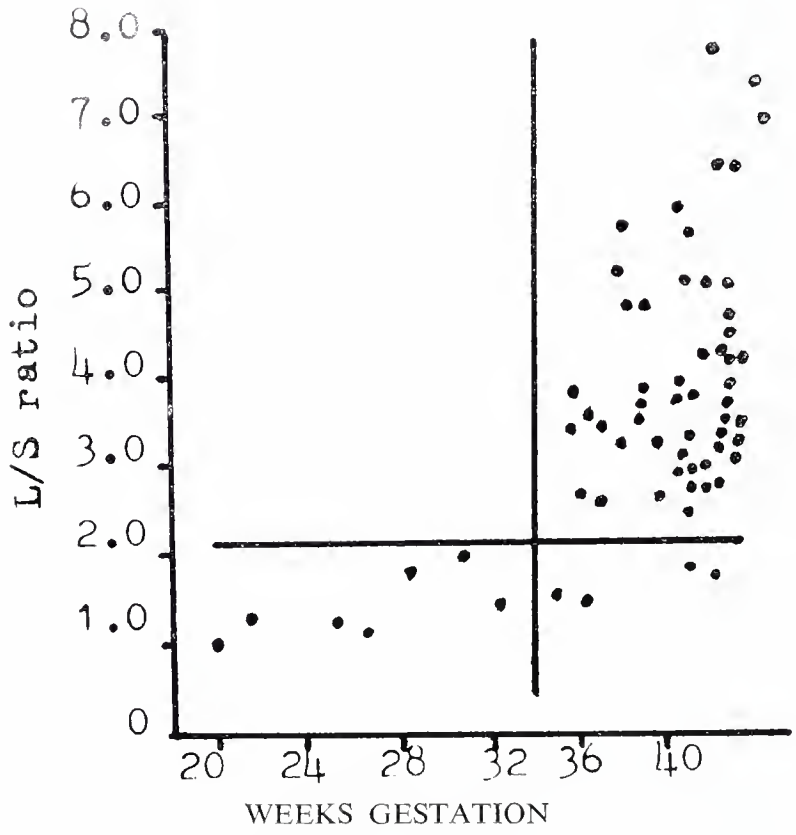
The amniotic fluid is centrifuged at 2,000 r.p.m. for 30 minutes. To 5 ml. of amniotic fluid supernatant in a 50 ml. extraction tube, an equal volume of methanol and 10 ml. of chloroform are added in that order. The mixture is shaken for 30 seconds, and centrifuged at 2,000 r.p.m. for 10 minutes. As the mixture stands, an upper aqueous layer and a lower chloroform layer are formed often separated by a white interface containing lipoprotein. The aqueous layer and interface are discarded. The chloroform extract, containing the phospholipids, is then rewashed by adding 1/5 volume of 0.5% sodium chloride followed by vigorous shaking and centrifugation at 2,000 r.p.m. for 5 minutes; after standing the upper aqueous layer is discarded and the washed chloroform extract dried under nitrogen at 37°C. The Gluck method[16] now calls for precipitating the phospholipids with cold acetone, however, in the Kling method[7] acetone is not added and the residue is resuspended by the addition of 10 drops of chloroform. The Kling method thus is able to yield total lecithin while the Gluck method yields only an acetone precipitate.

Spellacy and Buhi in their study[1] reported that the phospholipid ratio remains stable when amniotic fluid is centrifugated immediately and cooled. It may be kept refrigerated at 4°C. for 12 hours or frozen at – 20°C for 6 months with no significant change in the result.

The suspension is then spotted on a T.L.C. plate formed with Eastman chromogram silica gel strips or Brinkman Silplate – 52 and chromotographed. The plate is then stained with Bromthymol blue detector and ammonium hydroxide solution is added. The height and width of the lecithin and sphingomyelin spots are measured with microcalipers, and the ratio of the results then calculated. (There is a slightly more accurate method described in which a Beckman densitometer equipped with a disc integrator reads the T.L.C. plates and calculates the total L/S ratio, but this apparatus is only available in large medical centre labs).

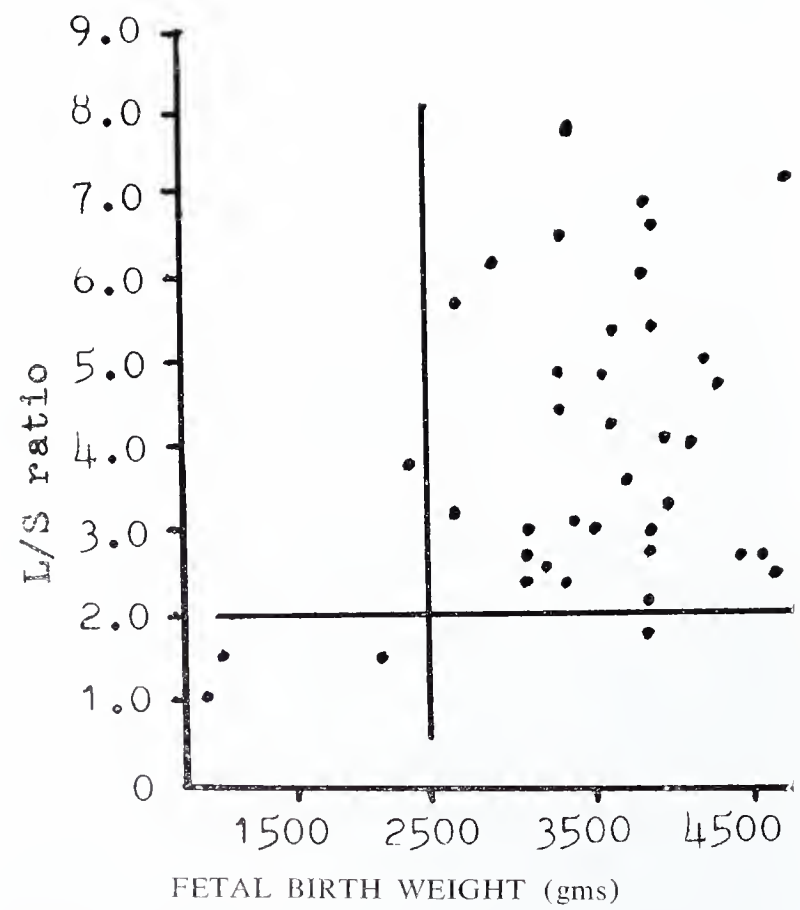
The results of the Kling study[7] are represented in figure III and figure IV.

FIGURE III




Total L/S ratios obtained at various stages of gestation. Kling et al[7]. (revised).

FIGURE IV




Comparison between total L/S ratio and fetal birth weight. All amniotic fluids were obtained within 48 hours of delivery. Kling et al[7]. (revised).

As indicated in figure IV the L/S ratio rises sharply at approximately 34 weeks of gestation and continues to rise until term. When an L/S ratio of 2:1 is correlated with a gestational age of 34 weeks or greater, false positives occur in only 2% of cases. Similar results have been reported by Gluck et al[16], Bhagwanani et al[18], and Spellacy and Buhi[1]. Therefore it can be assumed that if the lab reports an L/S ratio of 2:1, the foetus has the lung maturity of a normally developed 34 week foetus or older. As suggested in figure V when the L/S ratio exceeded 2:1 the birth weight (when the foetus was delivered within 24 hours of amniocentesis) was greater than 2500 gm. in 95% of cases.

The most important information derived from this study is the correlation of the L/S ratio with the incidence of the immature lung syndrome. R.D.S. did not occur when the L/S ratio was 2:1 or greater. However, when amniotic fluid analysis yielded an L/S ratio less than 2:1, 50% of these babies developed immature lung syndrome and 60% of these died within 24 hours of birth. 40% eventually recovered. Since these results are easily reproducible[1, 16, 19], there can be little doubt as to the accuracy of the L/S ratio in predicting the probability of foetal survival. It must also be stressed that the L/S ratio is a direct measurement of foetal lung maturity and not just an indication of gestational age from which lung maturity may or may not be accurately predicted. As stated earlier up to 75% of neonatal deaths are due to R.D.S.[1] With the status of lung maturity known, all other tests would seem superfluous.

Another very important finding confirmed by the Kling study[7] is that the accuracy of the L/S ratio as an indicator of foetal maturity is not affected by maternal complications including toxemia, hypertension, Rh – isoimmunization, and diabetes. Because these diseases are the major factors that lead the obstetrician to consider early delivery, this finding is truly encouraging. Thus it would seem that the L/S ratio is indeed an excellent indicator of foetal lung maturity and a very valuable technique to be performed when a high risk obstetrical case dictates pregnancy intervention.

The original T.L.C. method as described by Gluck et al[16] is the most widely used method of determining the L/S ratio. Other methods of quantitating pulmonary phospholipids in utero have recently been described, and these promise to be much faster and easier to perform, without sacrificing the accuracy of the T.L.C. technique. Two such methods are the colorimetric analysis technique of Gusdon and Waite[20] and the "Shake Test"[21].

The "Shake Test" is worthy of further consideration. It is very simple to perform, no laboratory facilities are required, only minimal cost is involved, and it yields an estimation of foetal lung maturity within

minutes of amniocentesis. I must confess that I am prejudiced in acclaiming the merits of this technique, as I have worked in the Obstetrics unit in which this procedure was developed. I have performed this technique myself, and cannot overemphasize its simplicity and speed.

The "Shake Test" was developed at the University of California Medical Centre in San Francisco and appeared in the literature in May, 1972[21]. The test is based on the fact that pulmonary surfactant has the ability to form stable surface films that can support foam for long periods of time. Other substances in the amniotic fluid such as salts of free fatty acids, proteins and bile salts can also form this stable foam, so these must first be excluded from the surface films. This is accomplished by adding ethanol which acts as a nonfoaming competitive surfactant. It has been shown that at a surface tension of 29 dynes per centimeter (which corresponds to a volume fraction of 47.5% ethanol), only double-chain phospholipids should compete for surface film and thus allow stable foam formation. Therefore, if an equal volume of ethanol is added to the amniotic fluid in a test tube and stable foam appears after shaking, then the source of the foam must be double-chain phospholipid. A tube is considered positive if after 15 minutes the fluid supports a complete ring of small stable bubbles. (For an exact description of the original method and information concerning semiquantitative analysis see reference 21).

It must be mentioned that in certain pathological situations false positives may occur. Blood meconium, and ascitic fluid all contain sufficient amounts of double-chain phospholipids to form a stable ring of foam in the presence of 47.5% ethanol. This is the major limitation of the "Shake Test".

The results of this study correlate well with the L/S ratio. There is also a correlation of the positive "Shake Test" and foetal lung maturity (as assessed by the absence of the immature lung syndrome) that is as definitive as the correlation produced by the Gluck method[16] when considering an L/S ratio equal to or greater than 2:1. Thus, this test seems an adequate substitute for T.L.C. when the immediacy of the situation or the lack of equipment make the T.L.C. impossible.

## CONCLUSION

Several studies have been done comparing the accuracy of the various methods of estimation of foetal maturity described above. Theoretically since R.D.S. is the cause of the majority of neonatal deaths, phospholipid studies would seem to be the most accurate tests in predicting foetal survival. The comparative studies of Spellacy and Buhi[1], and Kling et al[7] yield undisputable results lending practical support to this theoretical supposition. These studies also suggest that while phospholipid studies are definitely the most accurate, creatinine studies are also very useful in estimating foetal maturity. In addition Kling[7] suggests that when both determinations are made and correlated an estimation of even greater certainty is possible. An illustration of this follows from the Kling et al study[7].

If only the creatinine value is considered, neonatal death occurred in 18% of those cases in which the amniotic fluid creatinine concentration was less than 1.75 mg.%. If only the L/S radio is considered, 33% of cases with L/S ratio less than 2:1 died. But if the cases with a creatinine concentration of less than 1.75 mg.% plus an L/S ratio less than 2:1 are examined, a neonatal mortality rate of 50% is revealed. Thus combining the results of these tests whenever possible yields the most accurate prediction of foetal survival.

The fat-laden cell determinations and bilirubin colorimetric results have proved quite unreliable when compared with creatinine and phospholipid studies.

Thus, in recent years the many new investigations on amniotic fluid have added much to the armamentarium of the obstetrician when dealing with high risk obstetrics. When early termination of pregnancy is considered, he not only has a reasonable idea of maternal outcome, but can also predict the chances of survival of his other patient, the foetus.

## REFERENCES

1) Spellacy, W. N.; Buhi, W. C.: Amniotic fluid lecithin sphingomyelin ratio as an index of fetal maturity. Obstet. & Gynec. 39: 852-60, June, 1972.
2) Underhill, R. A.; Beazley, J. M.; Campbell, S.: Comparison of ultrasound cephalopelvimetry, radiology, and liquor studies, in patients with unknown confinement dates. Br. M. J. 3: 736-8, 1971.
3) Bross, I. D. J.; and Nachimethu, M. S.: Leukemia from low level radiation, identification of susceptible children. N. E. J. M. vol. 287, no. 3, July 20, 1972.
4) Wagatsuma, T.: Intraamniotic injection of saline for therapeutic abortion. Am. J. Obstet. & Gynec. 93: 743-5, Nov. 1965.
5) Bevis, D. C. A.: Composition of liquor amnii in hemolytic disease of the newborn. Lancet 2: 443, 1950.
6) Gordon, H.; Brosens, I.: Cytology of amniotic fluid: A new test for fetal maturity. Obstet. & Gynec. 30: 652-6, 1967.
7) Kling, O. R.; Crosby, W. M.; Merrill, J. A.: Amniotic fluid correlates of fetal maturity and perinatal outcome. Gynec. Invest. 4: 38-49, 1973.
8) Liley, A. W.: Liquor amnii analysis in the management of the pregnancy complicated by rhesus sensitization. Am. J. Obstet. & Gynec. 82: 1359-70, Dec. 1961.
9) Mandelbaum, B.; Lacroix, G. C.; Robinson, A. R.: Determination of fetal maturity by spectrophotometric analysis of amniotic fluid. Obstet. & Gynec. 29: 471-4. 1967.
10) Pitkin, R. M.; Zwirek, S. J.: Amniotic fluid creatinine. Am. J. Obstet. & Gynec. 98: 1135-9, Aug. 1967.
11) Doran, T. A.; Bjerre, S.; Porter, C. J.: Creatinine, uric acid, and electrolytes, in aminotic fluid. Am. J. Obstet. & Gynec. 106: 325, Feb. 1970.
12) Owen, J. A. et al. The determination of creatinine in plasma or serum and in urine; a critical examination. Biochem. J. 58: 426-37, 1954.
13) Technicon Corporation, Ardsley, New York. Method 11B for AutoAnalyzer. 1965.

*(Continued on page 69)*

# *Alpha$_1$ Antitrypsin Deficiency*

*Doug Cochrane, 7T5*

## INTRODUCTION

Alpha$_1$ antitrypsin ($A_1AT$) deficiency is a genetically determined inborn error of metabolism which is associated with hepatic cirrhosis and early progressive chronic obstructive lung disease (C.O.L.D.).

The interest in $A_1AT$ deficiency stems from the work of Laurell in 1963[7]. He noticed the absence of the alpha$_1$ globulin peak on the occasional serum electrophoretic pattern. With the aid of Erikson, he discovered that this pattern was seen in the serum of adult patients with pulmonary emphysema. Erikson, in further follow-up of these patients and their families, came to the conclusion that the deficiency of the $A_1$ globulin peak was genetically determined and was associated with a pre-disposition to the development of emphysema.

Subsequent work by Sharp in 1969 established an association between an identical electrophoretic pattern and the appearance of hepatic cirrhosis in children.

## BIOCHEMISTRY AND GENETICS

Earlier investigators[8] had shown that the alpha globulin fraction of serum proteins was largely composed of anti-enzymes capable of inhibiting proteases (e.g. trypsin) from various sources. 90% of the serum inhibiting power was associated with the alpha$_1$ globulin fraction and 10% with the alpha$_2$ fraction. It was therefore natural to name the deficiency state alpha$_1$ antitrypsin deficiency. It was also noted that the protease inhibiting capacity of serum was increased during infections, pregnancy and in patients on oral contraceptives.

Biochemically, $A_1AT$ is a low molecular weight glycoprotein having the ability to inhibit, in vivo and in vitro, elastase, collagenase, trypsin, chymotrypsin, thrombin and plasmin as well as various plant and bacterial proteases. The anti-enzyme itself can be inactivated by some organisms, especially the gram negatives[9].

Genetic studies have revealed that the inheritance of $A_1AT$ serum deficiency is controlled in an autosomal recessive manner.

The diagnosis of $A_1AT$ deficiency in children with jaundice or hepatomegaly of unknown origin, or in adults with early progressive C.O.L.D., is relatively simple. On cellulose acetate serum electrophoresis, the alpha$_1$ globulin peak is low in those whose serum is deficient. In order to assess the serum levels of $A_1AT$, measurements are made of serum trypsin or elastase inhibiting power. In measuring serum inhibiting power, purified enzyme and its substrate are mixed with the patient's serum. The concentration of $A_1AT$ can be measured by radial immuno-diffusion, using reliable standards and a specific anti-$A_1AT$ antibody.

Such studies have revealed three detectable levels of serum anti-enzyme activity[8]:

*Normal Concentration:* – 200 mg./100 ml. serum, serum trypsin inhibiting capacity (STIC) 0.85 u.

*Intermediate Deficiency Concentration:* – 60-190 mg./100 ml., STIC 0.4-0.85 u.

*Severe Deficiency Concentration:* – 60 mg./100 ml., STIC 0.4 u.

Complicating the assessment of individuals with $A_1AT$ deficiency is the fact that some 20 variants of the $A_1AT$ molecule are now recognized. These genetic alleles have been grouped by Fagerhol[4] into a system which he designated as Pi (Protease inhibitor). To determine an individual's genotype, Pi typing is carried out. This is a complex 2-step procedure involving an initial starch gel electrophoresis which separates the $A_1AT$ serum variants on the basis of electrophoretic mobility. A second electrophoresis using agarose gel containing a specific anti-$A_1AT$ antibody is then carried out at right angles to the first. This produces a series of precipitation peaks.

Based on the electrophoretic mobility of the protein component, the responsible genes have been named. The principle normal gene is designated $Pi^M$ while the gene commonly associated with serum deficiency is $Pi^Z$. Decreasing electrophoretic mobility is reflected by higher alphabetical designations. The lowest serum levels of $A_1AT$ are found in the homozygote ZZ genotype. Another relatively common variant is associated with the S allele. The SS homozygote exhibits an intermediate deficiency of $A_1AT$ as does the heterozygote MZ. Only the Z and S alleles have been shown to be associated with disease. It is felt that the Z allele as well as resulting in lower serum levels of $A_1AT$ activity also produces an abnormal $A_1AT$ protein.

Lieberman and associates[8] have discovered that patients with abnormal $A_1AT$ variants also have in their serum a second antigenic protein apparently derived from the $A_1AT$ molecule. In such cases a double ring pattern is formed on radial immunodiffusion using a specific antibody prepared with acid denatured $A_1AT$. The presence of this antigen in a patient's serum suggests that the serum contains denatured $A_1AT$ and that the abnormal variants have an increased tendency to denature in vivo.

Recent work by Bell and Carrel[2] has shown that the $A_1AT$ of the ZZ genotype could be produced by the partial removal of sialic acid from normal MM $A_1AT$.

Epidemiological studies on adult patients have revealed that the Z allele is most commonly found among those of Northern and Central European background and is lowest among the American Negro and Indian and those of Jewish or Italian extraction.

Among the first group the incidence of the heterozygote deficiency is estimated at 4.7-9%.

## CLINICAL MANIFESTATIONS

Historically, serum $A_1AT$ deficiency was associated with familial panacinar emphysema in adults and hepatic cirrhosis in children. Characteristically the lung disease presents at an earlier age than would normally be expected (av. of 43 yrs.). Recurrent lower respiratory tract infections may or may not have been present. Commonly the patient complains of progressive dyspnea with minimal cough or respiratory infection. He or she usually has little or no smoking history. The chest film shows a decrease in basilar vascular markings with or without bullous formation and a higher than normal position of the pulmonary arteries in the hila. According to Lieberman[8], approximately 26% of all emphysematous patients tested at the Long Beach V.A. Hospital, and at the City of Hope Medical Center had either severe or intermediate deficiency. The prevalence of $A_1AT$ deficiency increased with younger patients, reaching approximately 50% in those patients with clinical C.O.L.D. by 50 years of age or less. It was concluded that the $A_1AT$ deficiency predisposes to an earlier onset of emphysema and a decreased life expectancy.

Significant differences between the severe and intermediate deficiency states were noted. The severely deficient patients had an earlier onset of clinical disease ($45 \pm 8$ yrs. vs. $57 \pm 8$ yrs. for those with intermediate deficiency) and an earlier onset of symptoms ($28 \pm 11$ yrs. vs. $47 \pm 10$ yrs.). As well, the severely deficient patients smoked significantly less.

Sharp[9] was the first to establish an association between serum $A_1AT$ deficiency and liver cirrhosis in children. In his original study and those done at the Hospital for Sick Children in Toronto, most of the patients presented within their first year of life. Cholestatic jaundice, hepatomegaly with or without splenomegaly and failure to thrive were the principal presenting features .The jaundice generally resolved by 6 to 8 months of age although organomegaly and abnormal liver function tests persisted. Gradually the complications of progressive cirrhosis became manifest (oesophageal varicies, ascites, respiratory infections especially pneumococcal). In Sharp's study patients died at approximately 3 years of age.

In a prospective study currently in progress at the Hospital for Sick Children (H.S.C.), patient death has resulted from hepatic failure, variceal hemorrhage and sepsis. The uniformly grave prognosis of early childhood death suggested by Sharp's study has not been consistently observed among the H.S.C. patients. Most patients have gone on to make a reasonable clinical recovery from the jaundice and hepatic dysfunction. Death due to complications of cirrhosis has occurred at ages 9-12 years. One patient did not exhibit the severe neonatal findings but did go on to develop progressive cirrhotic complications and died at 5 years.

In recent study from H.S.C.[5], it was pointed out that both liver cirrhosis and pulmonary emphysema could co-exist in the same patient. The liver disease presented in the manner described above. Pulmonary involvement was manifested by recurrent lower respiratory tract infections and pulmonary function abnormalities indicative of an obstructive defect. The patient, by age 10, had clubbing, increased A-P chest diameter and numerous basilar bullae were seen on X-ray. Right ventricular hypertrophy was indicated by the ECG tracing. Death occurred at 11.5 years, following a massive intraperitoneal hemorrhage presumed secondary to a ruptured splenic varix.

## PATHOLOGICAL MANIFESTATIONS

At necropsy the lung shows a uniform panacinar emphysema. Bullae may be present and are of variable size. Bronchial and bronchiolar dilatation without hypertrophy is present. Alveolar walls are stretched and disrupted. Retraction of broken wall elastica produces knobs in areas where the alveolar walls are discontinuous. With elastic stains, bronchial and bronchiolar walls show little or no elastica. The internal elastic lamina if present is attenuated, frayed or discontinuous (Fig. 1).

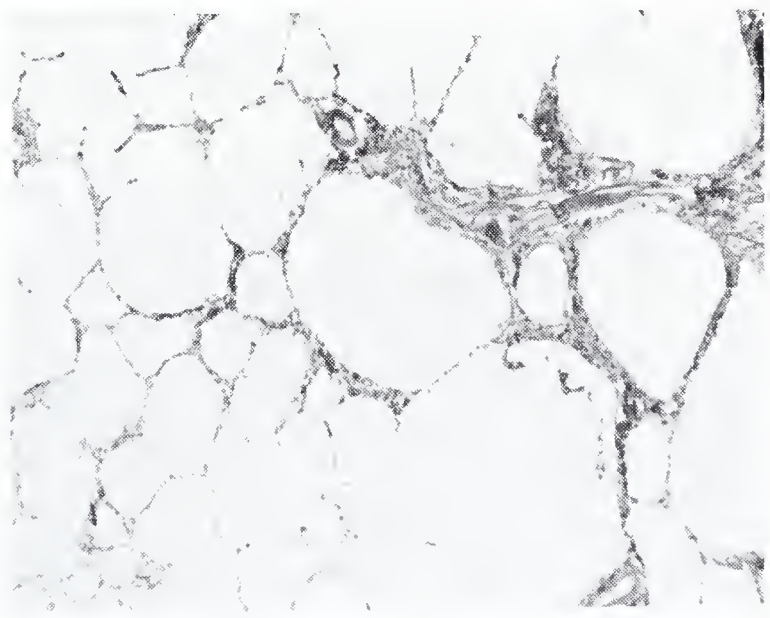


Fig. 1. Panlobular emphysema in $A_1AT$ deficiency. Magnification 14X

The hepatic lesions are characteristically found in patients with the X allelic variant. At autopsy, the liver is shrunken and displays micro and macronodules. There may be some bile staining and there is usually fatty change. On cutting, the liver is firm and the exposed surface reveals grossly visible nodules and extensive fibrosis. The extrahepatic biliary system is usually patent. Microscopically, the liver shows irregular nodules with extensive periportal fibrosis. Necrosis and regeneration are evident. Bile stasis is not generally prominent and is mainly intracellular. Bile duct prolif-

eration appears more reactive than primary. In some portal areas a chronic inflammatory cell infiltrate is seen (Fig. 2).

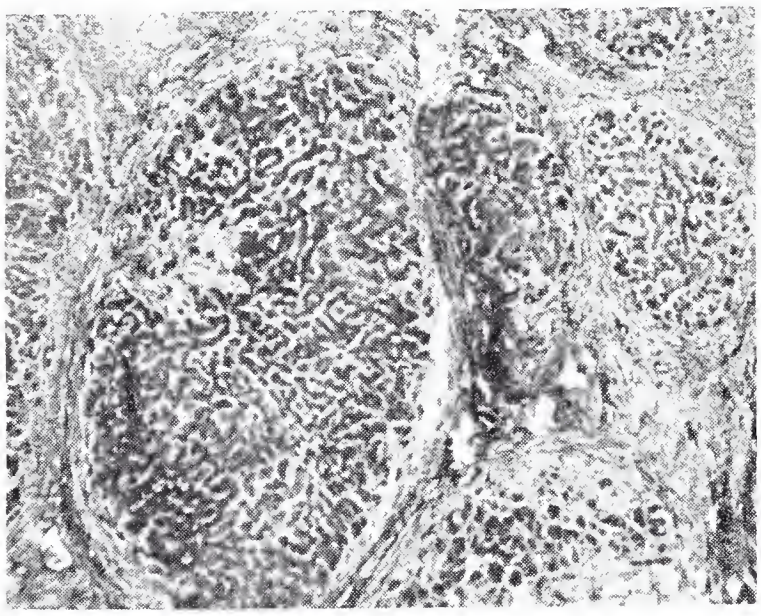


Fig. 2. Photomicrograph of an autopsy liver showing extensive cirrhotic involvement.
Magnification 14X

With H&E staining the periportal hepatocytes show typical pleomorphic, eosinophilic, intracytoplasmic inclusions characteristic of $A_1AT$ deficiency. The inclusions stain brick red with Masson and with PAS after diastase treatment, and dark blue with PTAH (Fig. 3). The material contained in the inclusions is stable in preserved tissue and therefore allows one to make a retrospective diagnosis based on the presence or absence of hepatocellular inclusions in postmortem cases of cirrhosis of unknown etiology.

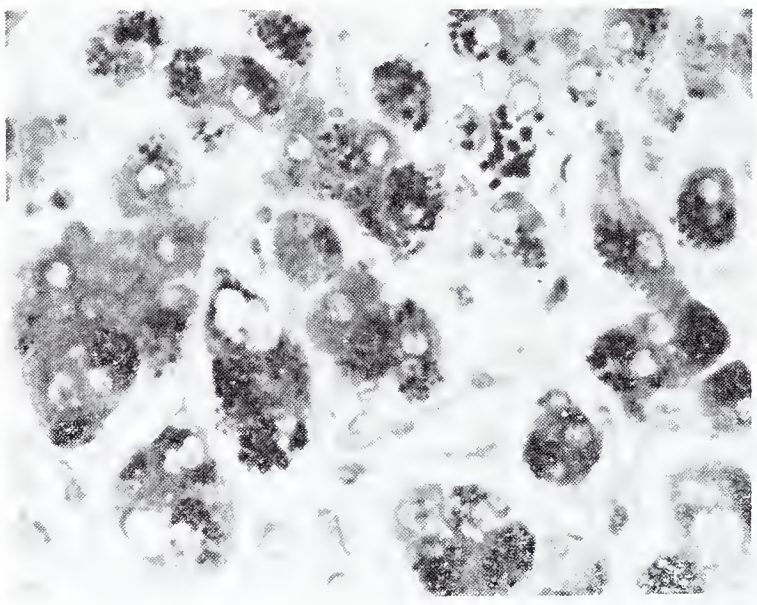


Fig. 3. Typical PTAH positive intracytoplasmic inclusions of $A_1AT$ deficiency.
Magnification 120X

When liver biopsy specimens and fresh autopsy material are incubated with labelled anti-$A_1AT$ antibody the inclusions are shown to be immunologically consistent with $A_1AT$. Often the diagnosis is made by immunofluorescence techniques when intracellular inclusions are not visible under the light microscope.

Electron microscopy shows the inclusions to lie within a single unit membrane studded with ribosomes. The $A_1AT$ itself appears as an amorphous material although Delellis et al[3] have described a microfibrillar substructure.

It should be noted that all patients who show evidence of serum $A_1AT$ deficiency in childhood may not initially have the visible inclusions. Serial biopsy specimens will show the inclusions as the child gets older, and that they increase in size with age. In such cases where the morphology does not include the typical inclusions, the histology reveals a picture reminiscent of neonatal hepatitis.

In adults who have only clinical lung disease, the liver at biopsy or at necropsy usually shows the characteristic inclusions. There are no such inclusions in adult emphysematous patients with normal serum $A_1AT$ levels.

In some of the autopsy cases at H.S.C., membranous proliferative glomerulonephritis has been observed. The significance of these lesions in the overall disease process is not known.

## PATHOGENESIS

The pathogenesis of the lung and liver lesions is still open to question. It is felt that the characteristic panacinar emphysema results from the inability of the patient's serum to inactivate leukocytic and macrophagic proteases released in inflammatory exudation. Normally such endoproteases (one of which is elastase) serve to digest the inflammatory debris within the exudate and are prevented from attacking alveolar walls by the presence of $A_1AT$. With a deficiency of this anti-enzyme, the endoproteases are free to digest the alveolar walls. The host's defense reactions to recurrent or chronic infections could thus overwhelm any existing $A_1AT$ and result in the development of panacinar emphysema.

The normal lung serves as a leukocyte storage depot. In the erect lung, the number of leukocytes stored in the bases of the lungs is greater than in the apices simply because of the pattern of regional blood flow. These facts could account for the more severe basilar involvement.

Animal studies using inhaled aerosals of the proteolytic enzyme papain have produced panacinar emphysema. This is felt to indicate the role of proteolytic enzymes in the pathogenesis of the lung lesions.

The role of $A_1AT$ deficiency in the development of liver cirrhosis is less well defined. The accumulation of $A_1AT$ in the rough endoplasmic reticulum may reflect a genetic alteration in the carbohydrate moiety of the molecule which prevents its normal transport out of the cell. A small amount would be expected to escape by passive diffusion and would therefore

account for the fact that complete absence of serum $A_1AT$ is not found in the ZZ genotype[2].

How does low serum $A_1AT$ lead to cirrhosis? Speculation has been that the lack or near lack of the anti-enzyme may render the liver parenchyma more susceptable to noxious agents. Studies at H.S.C. have failed to reveal any increased incidence of Australian Ag, lues, cytomegalovirus or toxoplasmosis in patients with $A_1AT$ deficiency liver disease. Perhaps the reactive fibrosis secondary to hepatic inflammation is not resolved in the absence of $A_1AT$. A storage disease type mechanism may also play a role.

The importance of $A_1AT$ deficiency is the severe morbidity and mortality it produces. Presently there is no cure although liver transplantation has been used in one case[9]. The recipient's $A_1AT$ serum levels were normal in 2 days and remained normal until his death secondary to respiratory complications. Attempts to increase serum $A_1AT$ levels with diethylstilbestrol[9] and other drugs have failed.

Since $A_1AT$ deficiency is a hereditary disease, genetic counselling of parents and the assessment of familial $A_1AT$ patterns become critically important. Sharp estimates the incidence of severe $A_1AT$ deficiency to equal that of cystic fibrosis[9]. Prenatal diagnosis via the measurement of $A_1AT$ levels in amniotic fluid is currently being studied[11]. Adults with the condition must be encouraged to give up smoking and to avoid respiratory irritants.

*ACKNOWLEDGEMENT*

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REFERENCES

1) Aagenaes, F.; Matlary, A., et al: Neonatal Cholestasis in Alpha$_1$ Antitrypsin deficient children. Acta. Paediat Scand. 61: 632-642, 1972.
2) Bell, O. F., and Carrell, R. W.: Basis of the Defect in Alpha$_1$ Antitrypsin Deficiency. Nature 243: 411-412, 1973.
3) Delellis, R. A., et al: Distinctive Hepatic Cell globules in adult Alpha$_1$ Antitrypsin Deficiency. Med. Clin. North America 85: 308-316, 1972.
4) Fagerhol, M. K.; Laurell, C. B.: The Pi system of inherited variants of serum alpha$_1$ antitrypsin. Prog. Med. Genetics 7: 96-111, 1970.
5) Glasgow, J. F., et al: Alpha$_1$ Antitrypsin Deficiency in association with both cirrhosis and chronic obstructive lung disease in two sibs. Am. J. Med. 54: 181-144, 1973.
6) Gordon, H. W., et al: Alpha$_1$ Antitrypsin accumulation in livers of emphysematous patients with $A_1AT$ deficiency. Human Pathology 3: 361-370, 1972.
7) Laurell, C. B.; Erikson, S.: Electrophoretic Alpha$_1$ globulin pattern of serum alpha$_1$ antitrypsin deficiency. Scand. J. Clin. Lab. Invest. 15: 132-140, 1963.
8) Lieberman, J.: Alpha$_1$ Antitrypsin Deficiency. Med. Clin. North America 57: 691-707, 1973.
9) Sharp, H. L.: Alpha$_1$ Antitrypsin Deficiency. Hospital Practice 6: 83-96, 1971.
10) Talamo, R. C., et al: Symptomatic pulmonary emphysema in childhood associated with hereditary alpha$_1$ antitrypsin and elastase inhibitor deficiency. Journal of Pediatrics 79: 20-26, 1971.
11) Wilkinson, E. J., et al: Alpha$_1$ Antitrypsin in amniotic fluid. Lancet 1: 559, 1973.

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14) Clements, J. A.: Surface tension of lung extracts Proc. Soc. Exp. Biol. Med. 95: 170-2, 1957.
15) Brumsley, G. W.; Hodson, W. A.; Avery, M. E.: Lung phospholipids and surface tension correlations in infants with and without hyaline membrane disease and in adults. Pediat. 40: 13-19, July, 1967.
16) Gluck, L. et al. Diagnosis of the respiratory distress syndrome by amniocentesis: Am. J. Obstet. & Gynec. 109: 440-5, 1971.
17) Morgan, T. E.: Pulmonary Surfactant. N. E. J. M. 284: 1185-93, 1971.
18) Bhagwanani, S. G.; Fahmy, D.; Turnbull, A. C.: Prediction of neonatal respiratory distress by estimation of amniotic fluid lecithin. Lancet ii: 159-62, 1972.
19) Gauthier, C. J.; Desjardins, P.; McLean, F.: Fetal maturity: amniotic fluid analysis correlated with neonatal assessment. Am. J. Obstet. & Gynec. 112: 344-50, 1972.
20) Gusdon, J. P. Jr.; Waite, M. B.: A colorimetric method for amniotic fluid phospholipids and their relationship to the respiratory distress syndrome. Am. J. Obstet. & Gynec. 112: 62-71, Jan. 1972.
21) Clements, J. A.; Platzker, A. C.; Creasy, R. K.; Margolis, A. J., et al: Assessment of the risk of respiratory distress syndrome by a rapid test for surfactant in amniotic fluid. N. E. J. M. 286: 1077-81. May 18, 1972.

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# Case Report: Erythema Nodosum

*Branislav Belovic, M.D., M.Sc.**

*An unusual case of a patient with sarcoidosis presenting with erythema nodosum, bilateral hilar lymphadenopathy and inactive ulcerative collitis is reported. Diagnosis, course, therapy and prognosis of this patient are discussed.*

## INTRODUCTION

Erythema nodosum (EN) is a transitory syndrome consisting of acute, inflammatory, erythematous cutaneous nodules that are usually limited to the shins and less commonly to the thighs and forearms. Current thinking views EN as a "type reaction" occuring as a pattern of response to a wide variety of provoking agents.

In the case described herein, EN developed in a lady with sarcoidosis, manifested by bilateral hilar lymphadenopathy (BHL). As inactive ulcerative colitis was also present this case represents a rather rare combination of diseases.

## CASE REPORT

The patient, a 44 year old white woman, was admitted to the dermatology unit of Toronto General Hospital on July 3rd, 1973. She presented with a one month history of progressive development of painful "bumps" and swelling over both her lower legs and ankles. This was accompanied by intermittent chills and a low grade fever. One week prior to this admission she was examined elsewhere and given a single dose of a diuretic. The swelling largely subsided but the red nodules remained unchanged.

On functional inquiry she reported that her vision was good until approximately one year ago when she admitted having a "feeling of a foreign body" in her left eye and dryness in both eyes. She denied any heat or cold intolerance, palpitations, nervousness or tremor. During the past two weeks she developed an intermittent productive cough with small amount of yellowish sputum. She denied any hemoptysis, night sweats, chest pain, wheezing, orthopnea or paroxysmal nocturnal dyspnea. During the past year, however, she experienced a sudden onset of shortness of breath following fluid ingestion. Her appetite was good and she denied any recent weight loss, abdominal pain, change in bowel habits, or blood in the stool. She had an ileostomy in the past but had no recurrence of problems in this respect.

From her past history she claimed to have had a retrosternal goitre with tracheal compression. This was followed by a subtotal thyroidectomy in 1939. Ever since she has been euthyroid with no medication for thyroid replacement. Between 1964 and 1966 she was hospitalized three times for ulcerative colitis. In 1966 a subtotal colectomy with ileostomy was performed. This was followed in 1968 by the excision of a rectal stump affected by the ulcerative colitis. In 1968 she had a subconjunctival cyst removed from her left eye. She denied any past history of skin problems, tuberculosis, allergies or diabetes. Her family history was non-contributory.

Physical examination revealed a pleasant middle aged woman with an occasional hacking cough. Her vision and fundi were normal. There was a subconjunctival nodule, 2x3 mm in size, noted in the outer corner of her left eye. Her thyroid was not palpable. There was mild lymphadenopathy in the right axilla. Her chest was clear. A functioning colostomy opening was seen in the right lower abdominal quadrant. No organomegaly was detected by palpation. Findings on her skin were confined solely to both anterior lower legs and ankles. The lesions consisted of numerous dark red, slightly raised tender nodules, one to ten cm in diameter with indistinct borders (Fig. 1). Her scalp, hair and nails were normal. There was mild pitting edema of her lower legs and ankles. There were no skin lesions of sarcoidosis present on the rest of her skin. The remaining clinical examination was unremarkable.

Laboratory workup included basic and special investigations. Her Hgb was 10.5gm.%, Hct 32, WBC 7,500 with a normal differential. Sedimentation rate was 55 mm/hour. Urinaylsis was normal. Biochemical tests including serum GOT, LDH, bilirubin, blood sugar, BUN, creatinine, phosphorus and calcium, were all normal. Isotope assay for thyroid function was within normal limits. Serum protein electrophoresis showed slight decrease in albumin and slight increase in $\alpha_2$ globulin. Levels of serum IgG, IgM, IgA were normal. VDRL and ASOT were non-reactive. Delayed hypersensitivity skin testing showed a positive reaction to candida and mumps antigens, but was negative to tuberculin (10U and 25U), histoplasmin and coccidioidin antigen. The Kveim test employing the Siltzbach-Kveim antigen, was also done and checked six weeks following the intracutaneous injection of the antigen. This produced a seven mm. induration at the test site and biopsy was consistent with sarcoid granuloma. A throat culture was sterile. Sputum culture grew many oral bacteria and Candida albicans. Sputum smear and culture for acid fast bacilli was negative. Pulmonary function tests were normal. Chest X-rays revealed a bilateral hilar lymphadenopathy typical for sarcoidosis. Upper GI series was normal, apart from an ileostomy site. Her ECG was normal.

Following admission the patient was investigated and given bed rest with her legs elevated. She was not put on any medications. Over the next few days her nodules became less prominent. While in hospital she was seen by an ophthalmologist regarding a sub-

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conjunctival nodule in her left eye. From this it was suggested that the nodule represented an old scar from previous ophthalmic surgery rather than a sarcoid nodule. The patient was discharged on July 11th, 1973. One month following discharge the patient was free of any skin lesions although she continued to complain of occasional productive cough.

Fig. 1 ERYTHEMA NODOSUM ON ANTERIOR LOWER LEGS. Dark spot on the right shin is a biopsy site.

## DISCUSSION

This patient, who had ulcerative colitis in the past and was treated by proctocolectomy, developed erythema nodosum (EN) over her anterior lower legs. At the present time she lacks symptoms relating to the gastrointestinal system. Her ileostomy was functioning well and upper GI series was unremarkable. EN is known to be associated with exacerbations of ulcerative colitis[1], but this case was different in that the colon and rectum were entirely removed previously, and EN developed in the absence of any activity in the remaining gastrointestinal system. Thus, in this particular case, there is considerable doubt that EN developed on the basis of ulcerative colitis.

On routine chest X-rays a bilateral hilar lymphadenopathy (BHL) was found. This was without any involvement of lung parenchymal tissue. BHL in association with EN in symptomless patients with negative physical examination, should be regarded as a-priori evidence of sarcoidosis, according to Winterbauer et al[2]. Further evidence supporting the diagnosis of sarcoidosis in this patient is a positive Kveim test. Though recently there has been a lot of controversy about the specificity of the Kveim test, Siltzbach believes it is useful in the diagnosis of sarcoidosis[3]. With his Kveim test material he found that 90% of patients with BHL due to sarcoidosis are Kveim test positive, whereas less than 1% false positive reactions were being observed among patients with lymphadenopathy of nonsarcoid origin.

Further interesting findings in our patient were the presence of a subconjunctival nodule and dryness of the eyes. Although a biopsy was not performed it is possible that these were ocular manifestations of sarcoidosis. James has reported eye involvement in one third of patients with sarcoidosis[4]. He found keratoconjunctivitis sicca in 10 patients and conjunctival follicles in 5 patients out of 123 patients with eye involvement in sarcoidosis[4].

Although a mediastinal biopsy was not performed it is probable that this patient had sarcoidosis manifested by EN and BHL. Siltzbach, in a survey of 317 subjects with sarcoidosis, found that 55% of them presented initially with EN and 55% of them had BHL on initial chest X-ray examination[5].

The treatment of this patient consisted only of general measures. The lack of constitutional symptoms, the absence of mottling of fibrosis on chest X-rays, the absence of vital organ involvement (CNS, myocardium, kidney) and lack of hypercalcemia all suggest a good prognosis and therefore therapy with steroids was not indicated[6]. In patients with sarcoidosis, prednisone has been reported to be of more use in people with pulmonary parenchymal involvement[7].

The prognosis of this patient is probably good. Douglas[8], in his follow-up study of 86 cases with BHL associated with EN, lymphadenopathy, or eye involvement, reported spontaneous resolution of hilar glands in an average period of less than eight months. In only one case was there a recurrence of BHL before final resolution.

### REFERENCES


1. Johnson, M. L.; Wilson, H. T. H.: Skin lesions in ulcerative colitis. Gut, 10, 255-263, 1969.
2. Winterbaurer, R. H.; Belic, N.; Moores, K. D.: A Clinical interpretation of bilateral hilar lymphadenopathy. Ann. Intern. Med., 18: 65-71, 1973.
3. Siltzbach, L. E.: Bilaterial hilar adenopathy. Lancet, i, 879-880, 1973.
4. James, D. G.: The diagnosis and treatment of ocular sarcoidosis. Acta Med. Scand., Supplement 425, 176: 203-208, 1964.
5. Siltzbach, L. E.: "An International Kveim test study". Acta Med. Scand., Supplement 425, 176: 178-186, 1964.
6. Israel, H. L.: Steroid treatment in sarcoidosis. Acta Med. Scand., Supplement 42, 76: 297-298, 1964.
7. Israel, H. L.; Fouts, D. A.: A controlled trial of prednisone treatment of Sarcoidosis. Amer. Rev. Resp. Dis., 107: 609-614, 1973.
8. Douglas, A. C.: The prognosis of early sarcoidosis. Acta Med. Scand., Supplement 142, 176: 284-286, 1973.

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# *Grand Round Quiz — Clinical Biochemistry (Questions)*

Only one answer is correct for each question.

1. *In Tay-Sachs disease,*

   a) There is an accumulation of $GM_2$ ganglioside in brain tissue due to increased production of this substance.

   b) There is accumulation of a ganglioside of abnormal structure in brain tissue due to absence of a specific enzyme.

   c) There is accumulation of $GM_2$ ganglioside in brain tissue due to a deficiency of a $GM_2$ catabolic enzyme.

   d) Analysis of the patient's serum shows a total lack of hexosaminidase activity.

2. *You are investigating a patient suspected of having primary Type IIa hyperlipoproteinemia. You examine the serum electrophoresis pattern and a sample of fasting plasma that has been standing 0-4°C for 24 hours.*

   a) The plasma appears clear with a dense white floating layer. A single densely staining band is seen at the origin of the electrophoretogram.

   b) The plasma is totally clear. A normal electrophoretic pattern is seen save for a band of increased density at the beta position.

   c) The plasma is clear. A normal electrophoretic pattern is seen except for a broad dense band covering the beta and pre-beta position.

   d) The plasma sample is cloudy, resembling tomato soup. A perfectly normal electrophoretic pattern is seen.

3. *While discussing the problem of jaundice with a colleague, you make the following four statements, only one of which is correct.*

   a) Jaundice due to increased hemolysis results in an increase of the direct-reacting fraction of plasma bilirubin.

   b) The presence of increased urinary urobilinogen suggests an extrahepatic obstruction as a cause of jaundice.

   c) The presence of bilirubin in the urine is evidence of conjugated hyperbilirubinemia.

   d) Most of the normal serum bilirubin will react in the direct van den Bergh reaction.

4. *Glutamate Oxaloacetate Transaminase (GOT):*

   a) is not normally present in human serum.

   b) is present as at least two isoenzymes in many tissues except skeletal muscle.

   c) reaches peak levels in the blood 7-10 days following myocardial infarction.

   d) is an enzyme that catalyses the synthesis of amino acids from keto acids.

5. *Elevated levels of SGOT may be expected in all of the following conditions except one:*

   a) coronary insufficiency
   b) acute pancreatitis
   c) muscular dystrophy
   d) toxemia of pregnancy
   e) salicylate ingestion

6. *An adult male patient produced three successive 24 hour urine samples for biochemical testing. You determined the total creatinine contents and got the following values: 1375 mg, 825 mg, 1355 mg, respectively.*

   a) All three urine samples are useful since there is considerable variation in serum creatinine and therefore creatinine excretion.

   b) The second sample is not reliable since creatinine excretion is independent of urine flow and fairly constant from day to day.

   c) All three samples are unreliable since creatinine excretion is dependent on the amount of protein in the diet and this was not controlled.

   d) The second sample is not reliable because creatinine is neither secreted nor reabsorbed in the kidney and therefore the 24-hour creatinine excretion should be constant.

7. *Which of the following sets of blood chemistry results would be consistent with the chronic administration of a carbonic anhydrase inhibitor?*

   a) $HCO_3^-$ 13 meq/ml, $paCO_2$ 28 mm Hg., $Cl^-$ 115 meq/L., $K^+$ 2.8 meq/L.

   b) $HCO_3^-$ 24 meq/L., $paCO_2$ 30 mm Hg., $Cl^-$ 102 meq/L., $K^+$ 4.0 meq/L.

   c) $HCO_3^-$ 13 meq/L., $paCO_2$ 28 mm Hg., $Cl^-$ 90 meq/L., $K^+$ 2.8 meq/L.

   d) $HCO_3^-$ 30 meq/L., $paCO_2$ 40 mm Hg., $Cl^-$ 102 meq/L., $K^+$ 4.0 meq/L.

8. *A five year old boy is brought to hospital. Over the past month he has been restless, irritable, and has lost five pounds in weight. Recently he developed a stiff neck, some nausea and vomiting. To confirm your diagnosis of tuberculous meningitis you do a lumbar puncture. Which of the following CSF findings would be consistent with your diagnosis.*

   a) CSF pressure↑, cloudy fluid, total protein 60 mg%, glucose 65 mg%.
   b) CSF pressure↑, clear fluid, total protein 85 mg%, glucose 20 mg%.
   c) CSF pressure↑, clear fluid, total protein 12 mg%, glucose 20 mg%.
   d) CSF pressure↑→, clear fluid, total protein 12 mg%, glucose 85 mg%.

9. *A 50 year old woman presents at your office with progressive fatigue, depression, paresthesiae, and muscle weakness over a two year interval. On examination you discover that she has hyperreflexia and Trousseau's sign is positive. Some of the laboratory results are: serum calcium 5.4 mg%, serum phosphorus 8.6 mg%, BUN 25 mg%, serum creatinine 1.4 mg%, serum alkaline phosphatase 4 Bodansky units.*

   *On this basis you rule out all but one of the following:*

   a) osteomalacia
   b) hypoparathyroidism
   c) chronic renal failure
   d) steatorrhea

# *Grand Round Quiz — Clinical Biochemistry (Answers)*

1. – c)

Tay-Sachs disease is caused by a lack of hexosaminidase A in the patient's tissues. Hexosaminidase is an enzyme that takes part in the catabolism of $GM_2$ ganglioside. Absence of the A component of the enzyme results in the accumulation of $GM_2$ of normal structure in brain tissue. Hexosamininidase activity due to the B component of this enzyme can be detected in the patient's serum.

2. – b)

In primary type II hyperlipoproteinemia there is an increased concentration of the low density lipoprotein (LDL) fraction of plasma. The two subclasses of Type II are: IIa- normal VLDL concentration and IIb- increased VLDL concentration. Since this is type IIa and the patient has fasted the plasma will be clear and a dense band corresponding to an increased LDL will be seen at the beta position of the electrophoresis pattern.

3. – c)

Bilirubin results from the catabolism of hemoglobin. Most bilirubin in the serum is bound to albumin since it is not water soluble. This unconjugated bilirubin will not react in the direct van den Bergh reaction and therefore jaundice due to increased hemolysis will not show an increase in the direct-reacting fraction. Likewise since bilirubin is protein-bound it is not filtered by the kidney and therefore any bilirubin in the urine must be conjugated. Since there is a renal threshold for conjugated bilirubin the presence of urinary bilirubin suggests conjugated hyperbilirubinemia.

4. – d)

Glutamate-oxaloacetate transaminase catalyses the reversible transfer of an amino group from glutamic acid to oxaloacetic acid forming aspartic acid and αketoglutaric acid. It is present in all tissues and is normally found in serum at less than 50 Karmen units. It is released into the blood by damaged myocardium reaching peak levels 1-3 days after an infarct.

5. – a)

6. – b)

Creatinine is formed from phosphocreatine in the cytoplasm of muscle cells and diffuses into the plasma. The plasma levels are fairly constant and are related to muscle mass and are independent of diet. Creatinine excretion is independent of urine flow and therefore constant from day to day. Creatinine content is a good indication of the adequacy of 24-hour urine collection. In man creatinine is secreted into the kidney tubules and a small amount is passively reabsorbed.

7. – a)

Chronic administration of carbonic anhydrase inhibitors closely simulates the situation of renal tubular acidosis. There is an inability to appropriately acidify the urine leading to hyperchloremic metabolic acidosis and hyperventilation. There is no exchange of $H^+$ ion for $Na^+$ and a failure to reabsorb biacarbonate. Blood potassium levels are depleted since there is an increased exchange of $K^+$ for $Na^+$ in the renal tubules.

8. – b)

Tuberculous meningitis is seen most commonly in young children in the first year of infection. The usual findings are elevated CSF pressure, clear or slightly turbid fluid, increased total protein (N 20– 40 mg%) and decreased glucose (less than half the blood glucose).

9. – b)

Of the non parathyroid causes of hypocalcemia, osteomalacia and steatorrhea can be distinguished by a low or normal serum phosphorus since parathyroid hormone would attempt to maintain serum calcium and promote renal excretion of phosphorus. Serum alkaline phosphatase would be high in osteomalacia. Both hypoparathyroidism and chronic renal failure would have a high serum phosphorus value; however, there is no indication of renal failure in this case.

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